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*A THIRD ABNORMAL HEMOGLOBIN ASSOCIATED WITH HEREDITARY HEMOLYTIC ANEMIA**

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Communicated by Linus Pauling, October 26, 1951

Previous work from these Laboratories has established the existence of an abnormal hemoglobin in sickle cell trait and sickle cell anemia and has provided the basis for a theory relating the presence of the abnormal hemoglobin to the clinical pictures in sickle cell trait and sickle cell anemia.[1] It was shown in this work that the electrophoretic picture in these conditions is compatible with the usual genetic picture that both parents of an individual suffering from sickle cell anemia have the sickle cell trait.[2] There are in the literature, however, a number of reports in which one of the parents of a sickle cell anemia victim possesses non-sickling erythrocytes. In some cases of this type the parent whose cells do not sickle has been shown to have thalassemia minor (Cooley's trait), and hematologic studies on the erythrocytes of the anemic individual have revealed the presence of properties which are present in the erythrocytes both of sickle cell disease and of thalassemia.[3,4] In other cases the hemoglobin of the parent having the non-sickling cells has a greater electrophoretic mobility on the acid side of the isoelectric point than either normal hemoglobin or sickle cell hemoglobin, and the hemoglobin of the anemic child contains both this new hemoglobin and sickle cell hemoglobin.[5]

The present report deals with the identification of still another form of human hemoglobin in five members of a family in which the genetic picture is not typical of sickle cell anemia, although two of the members have in the past been diagnosed as having sickle cell anemia. An earlier study of this family disclosed that the two anemic children and the father, who was not anemic, had sickling erythrocytes while the mother, two sisters and two brothers of the anemic children had non-sickling erythrocytes and were not anemic.[6] The father, one brother and one sister were not available for the present study.

Experimental Methods.—(*a*) *Sickling tests.* The sodium dithionite,[7]

sodium metabisulfite[8] and moist seal[9] methods were employed. The tests were repeated on specimens which were negative for sickling.

(*b*) *Electrophoretic studies.* The methods described in previous reports from these Laboratories were employed.[1,10] The carbonmonoxyhemoglobins were studied.

(*c*) *Solubility studies.* The solubilities of the ferrohemoglobins prepared from the erythrocytes of the members of this family were determined in a series of concentrated phosphate buffers. The amorphous rather than the crystalline state was examined for reasons which will be discussed later. Similar studies were conducted on the ferrohemoglobins from normal, sickle cell trait, and sickle cell anemia individuals for control purposes. (1) *Materials:* Concentrated hemoglobin solutions were prepared and were dialyzed against distilled water. A potassium phosphate buffer having a total phosphate concentration of 2.8 molar and a pH of 6.8[11] was prepared from reagent grade crystalline potassium dihydrogen phosphate and dipotassium phosphate powder. (2) *Experimental method:* From 8.00 to 9.60 ml. of the concentrated phosphate buffer were delivered from a buret into 10-ml. volumetric flasks, and 100 mg. of sodium dithionite, $Na_2S_2O_4$, was added to the buffer in each flask. The flasks were then filled to the 10-ml. mark with aqueous hemoglobin solution or with the hemoglobin solution and distilled water. The contents of the flasks were mixed by manual shaking, and the precipitate was separated from the solution by centrifugation. Experiments were discarded unless a visible precipitate formed within a few seconds of the mixing. The absence of a precipitate indicates either undersaturation or supersaturation, the latter occurring in some cases when the hemoglobin is present in but small excess.[12] Following the centrifugation, which caused the excess solid hemoglobin to collect above the dense solution, the solution of hemoglobin in phosphate buffer was removed. Its concentration was determined spectrophotometrically immediately after saturation with carbon monoxide. The temperature of the solutions at the end of the centrifugations was $18° \pm 2°$ C. The ionic strength of each solution was calculated from the amounts of 2.8 molar phosphate buffer and sodium dithionite added. The amount of hemoglobin present in each of the equilibrium mixtures was calculated from the volume and concentration of the hemoglobin solutions added.

Results.—(*a*) *Sickling tests.* The erythrocytes of R. H., a male, and B. H., a female, the two individuals who were previously reported as being anemic, were positive for sickling, and virtually all of their red cells sickled. The erythrocytes of their mother, M. H., a brother, G. H., and a sister, H. H. D., did not sickle. Three different specimens of M. H.'s blood were drawn and tested over a six-month period; at no time was any sickling elicited in her cells in spite of numerous tests. These results are in agreement with those reported in the previous study of this family.[6]

(*b*) *Electrophoretic studies.* The electrophoretic behavior of the hemoglobins of the two sickling individuals in this study, R. H. and B. H., appears to be the same as that previously found in cases of sickle cell anemia. R. H.'s carbonmonoxyhemoglobin was analyzed both in phosphate buffer and in cacodylate buffer. The scanning diagram in phosphate buffer of pH 6.90 and ionic strength 0.1 is identical to that previously published,[1] and the mobility in this buffer is 0.34×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}. The mobility determinations of seven different specimens of sickle cell anemia carbonmonoxyhemoglobin in this buffer averaged 0.33×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}.[13] In cacodylate-sodium chloride buffer of pH 6.50 and ionic strength 0.1, the major component of the carbonmonoxyhemoglobins from both R. H. and B. H. has the mobility of sickle cell carbonmonoxyhemoglobin. About 6% of R. H.'s hemoglobin and 12% of B. H.'s hemoglobin are present in this buffer as a slow-moving component having the approximate mobility of normal carbonmonoxyhemoglobin. These patterns in cacodylate buffer are very similar to those found in some cases of sickle cell anemia.[5, 10] The scanning diagrams of the carbonmonoxyhemoglobins from M. H., G. H., and H. H. D. in the cacodylate buffer are indistinguishable from those obtained from individuals having sickle cell trait.[10] Normal hemoglobin and a hemoglobin component which has the same mobility as sickle cell hemoglobin are present. The percentages of the abnormal hemoglobin present are 42, 35, and 49%, respectively, for M. H., H. H. D. and G. H.

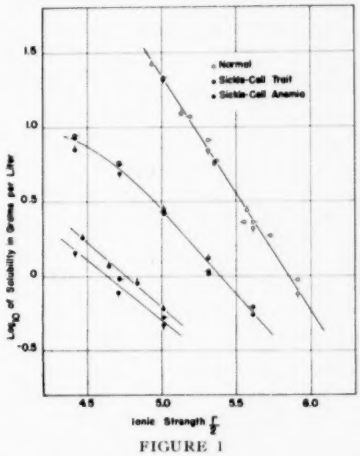

FIGURE 1

The solubilities in phosphate buffers at 18° C. of amorphous ferrohemoglobins from three normal individuals, three individuals having sickle cell trait and two individuals having sickle cell anemia. One of the normal specimens and the sickle cell anemia specimen having the lower solubility were examined twice and are represented by two series of points. Two curves are drawn for the sickle cell anemia points because the two specimens have slightly different electrophoretic patterns (see discussion section).

(*c*) *Solubility studies.* The results are indicated by figures 1 and 2. The points on the solubility curve for normal ferrohemoglobin represent experiments on three different specimens of normal hemoglobin, one of which was examined in two series of determinations. Specimens of hemoglobin from three individuals having sickle cell trait and two having sickle

cell anemia were examined. The anemia curve showing the higher solubility represents a single series of determinations on a sickle cell anemia hemoglobin specimen which shows on electrophoretic analysis approximately 16% of a slow component having the mobility of normal hemoglobin. The other anemia curve represents two series of determinations on a sickle cell anemia hemoglobin specimen having less than 5% of the slow component. The solubilities of the hemoglobins of the sickling members of the family being studied, R. H. and B. H., are between those of sickle cell trait individuals and sickle cell anemia individuals. The solubilities of the hemoglobins from the non-sickling individuals, M. H. and G. H., are nearly the same as those of specimens of normal hemoglobin. H. H. D.'s hemoglobin was not available in sufficient quantity to run the solubility experiments.

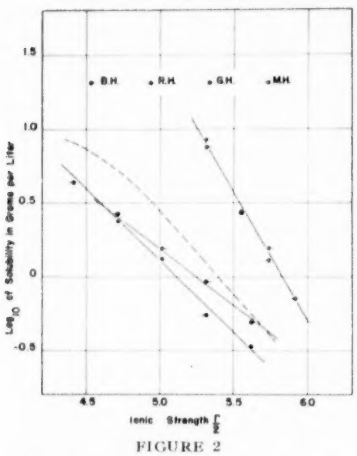

FIGURE 2

The solubilities in phosphate buffers at 18° C. of amorphous ferrohemoglobins from four members of the family under study. The dashed curve is the sickle cell trait curve from Figure 1. M. H. and G. H. have two-component electrophoretic patterns similar to those found in sickle cell trait. R. H. and B. H. have electrophoretic patterns with one major component, similar to those found in sickle cell anemia.

The solubility behavior of a single specimen of hemoglobin which contains 65% of normal hemoglobin and 35% of the new inherited abnormality reported by Itano and Neel[5] and of a single specimen of hemoglobin from an individual suffering from thalassemia major (Cooley's anemia) were investigated. The plot of the logarithm of the solubility against ionic strength for both these specimens fell within 0.1 logarithm unit of the plot for normal hemoglobin.

(*d*) *Summary of results.* The hemoglobins from M. H., G. H., and H. H. D. resemble the hemoglobins from individuals having sickle cell trait in their electrophoretic behavior but differ in having a greater solubility and in failing to cause sickling. Likewise, the hemoglobins from R. H. and B. H. resemble hemoglobins from individuals having sickle cell anemia in their electrophoretic behavior but differ in having a greater solubility.

Nomenclature.—In order to facilitate the discussion in the present paper and to avoid confusion in future works, it seems desirable at this time to establish a system of symbols for identifying the various forms of

adult human hemoglobin. Table 1 lists the characteristics of the four forms of adult human hemoglobin which have been identified to date. While the names normal hemoglobin and sickle cell hemoglobin adequately describe the first two forms and will be used in this paper, names of equally descriptive nature have not yet been proposed for the two newly recognized forms. Therefore for purposes of identification and discussion normal hemoglobin, sickle cell hemoglobin,[1] the abnormal hemoglobin reported by Itano and Neel,[5] and the abnormal hemoglobin reported in the present paper will be designated adult human hemoglobins *a*, *b*, *c* and *d*, respectively, more briefly as hemoglobins *a*, *b*, *c* and *d*. Mixtures of these hemoglobins which occur naturally may then be called hemoglobins *ab*, *ad*, *bc*, etc. Hemoglobins *c* and *d* have not been found free of other hemoglobins, and their behavior with respect to sickling and solubility are inferred from the behavior of mixtures of *c* and *d* with *a* and *b*. The solubilities of *c* and *d* are therefore not precisely known at this time, and they have been grouped together with that of normal hemoglobin as "high" with respect to that of sickle cell hemoglobin.

TABLE 1

CLASSIFICATION OF ADULT HUMAN HEMOGLOBINS[a]

NAME OF HEMOGLOBIN	IDENTIFICATION LETTER	CAUSES SICKLING	ELECTROPHORETIC MOBILITY[b]	SOLUBILITY[c]
Normal	*a*	No	Slow	High
Sickle cell	*b*	Yes	Intermediate	Low
...	*c*	No	Fast	High
...	*d*	No	Intermediate	High

[a] The hemoglobin present in individuals having thalassemia major is not included in this tabulation; some investigators are of the opinion that the abnormal properties of this hemoglobin are due to the presence of fetal hemoglobin.[29, 30] The possibility that the low mobility component in the hemoglobins of R. H., B. H. and certain sickle cell anemics may be fetal hemoglobin is being investigated.

[b] As carbonmonoxyhemoglobin in cacodylate-sodium chloride buffer of pH 6.50 and ionic strength 0.1. In this buffer each of these carbonmonoxyhemoglobins migrates as a positive ion.

[c] As amorphous ferrohemoglobin in concentrated phosphate buffers of pH 6.8 at 18°C.

Discussion.—The results of the sickling tests conducted on the individuals who were available for this study are in complete agreement with those found by the previous investigators of this family.[6] These workers noted in addition that the sickling was of the slow type in the sickling individuals, an observation which implies that the constitution of their erythrocytes is not identical with that of the fast sickling cells typically found in sickle cell anemia.

The observation that the non-sickling erythrocytes of M. H., G. H. and H. H. D. contain hemoglobin components[14] which migrate like those

found in sickle cell trait suggests two possible explanations. One explanation, that the membrane and internal structure of these cells are abnormally rigid and are able to withstand the tendency of the sickle cell hemoglobin to aggregate, is a difficult one to test unambiguously. The other explanation, that the abnormal component in these cells represents a hemoglobin which, while migrating with the same mobility as sickle cell hemoglobin, is actually a different molecular species, can be tested if an independent criterion for differentiating hemoglobins can be employed. Solubility differences exist among the hemoglobins of different species,[15] between adult and fetal hemoglobins[16] and between normal and sickle cell hemoglobins.[17, 18] The possibility that the abnormal hemoglobin in this family might have a characteristic solubility was therefore investigated.

Although in the majority of protein solubility studies crystalline protein preparations are used, this procedure was undesirable in the present work because the process of preparing crystals is likely to cause partial fractionation of the components.[19] Other undesirable features of solubility work on crystalline proteins are the length of time required for equilibration and the danger of supersaturation.[12] The low solubility of ferrohemoglobin in comparison with other compounds of hemoglobin[16] and the large difference in the solubilities of normal and sickle cell ferrohemoglobins suggested the use of this compound throughout the solubility experiments. Another advantage of using this compound is the very small dependence of its solubility on temperature.[16] The use of sodium dithionite served a double purpose; any ferrihemoglobin which forms during the preparation of the hemoglobin solutions is reduced to ferrohemoglobin by this compound, and no oxyhemoglobin can form in the presence of an excess of sodium dithionite because of the high rate of reaction of oxygen with this reducing agent. Both ferrihemoglobin and oxyhemoglobin are considerably more soluble than ferrohemoglobin, and their temporary presence might result in supersaturation. The amount of dithionite, while sufficient to maintain the hemoglobin[20] in the reduced, unoxygenated state, was small in relation to the total phosphate concentration of the buffer, so that its decomposition did not alter significantly the pH or the total ionic strength of the solutions. It was found that the equilibrium concentration of hemoglobin was attained within the time required to precipitate and separate by centrifugation the excess hemoglobin as amorphous solid. In the case of normal hemoglobin, presumably a one-component protein, the solubility of the amorphous phase was independent of the amount of the solid present. The solubility of sickle cell trait hemoglobin increased with the amount of the solid phase present, approaching a limiting value in the presence of a large excess of the two-component hemoglobin. Repeated centrifugation and resuspension of an amorphous precipitate of normal hemoglobin resulted in the formation of crystalline ferrohemo-

globin, which has a lower solubility than the amorphous form. The foregoing results, plus the reproducibility of the results as indicated by figure 1, indicate that amorphous hemoglobin possesses a metastable equilibrium solubility which is characteristic of the type of hemoglobin and of the concentration of the solvent buffer, and which is greater than that of the crystalline form. These results are in accord with those obtained by other workers[16,21] on the crystalline and amorphous hemoglobins of several species.

The actual solubility in moles per liter[22] of sickle cell trait hemoglobin falls much closer to that of sickle cell anemia hemoglobin than to that of normal hemoglobin although the percentage of sickle cell hemoglobin in sickle cell trait is always smaller than that of normal hemoglobin.[10] This indicates that, unlike the system treated by Northrop and Kunitz,[23] the amorphous solid solution of normal and sickle cell hemoglobin does not show a Raoult's Law type of behavior in its solubility but has a solubility much lower than that predicted from Raoult's Law. This finding precludes the determination of the composition of sickle cell trait hemoglobin in its two components from solubility data alone. The deviation of the sickle cell trait solubility data from a straight line in the high solubility region is undoubtedly due to the fact that in the experiments in this region the excess of hemoglobin present was small, and the composition of the solid phase was significantly altered by the solution of a large fraction of the added hemoglobin.

The observations that the solubilities of the two-component hemoglobins of M. H. and G. H. are nearly the same as those of normal hemoglobin and five to seven times as great as that of sickle cell trait hemoglobin, which has the same electrophoretic pattern, provide conclusive proof that the abnormal component in the hemoglobins of these individuals is not sickle cell hemoglobin. These results also suggest that, barring large interaction effects, the solubility of this abnormal hemoglobin is of the same order of magnitude as that of normal hemoglobin, which is about 14 times as soluble as sickle cell hemoglobin in the amorphous state. Thus the presence of a hitherto unreported hemoglobin component in the erythrocytes of M. H., the mother, G. H., and probably H. H. D. has been established without consideration of the data presented by the studies on R. H. and B. H. Probably the major electrophoretic component of the hemoglobins of the latter individuals contains two abnormal hemoglobins migrating with the same mobility. The presence of sickle cell hemoglobin is indicated by (1) the positive sickling test and relatively low solubility of their hemoglobins, (2) the identity of the electrophoretic mobility of the major component of their hemoglobins with that of sickle cell hemoglobin and (3) the presence of sickle cell trait in their father. The presence of the other hemoglobin, which henceforth will be called hemoglobin *d*, is indicated by (1) the greater solubility of their hemoglobins in com-

parison with sickle cell anemia hemoglobins having the same or very similar electrophoretic patterns and mobilities and (2) the presence of this hemoglobin in their mother and two siblings.

The explanation for the greater solubility of B. H.'s hemoglobin as compared to R. H.'s may lie in the electrophoretic result that B. H.'s hemoglobin has about 12% of a component having the mobility of normal hemoglobin while R. H.'s hemoglobin has only about 6% of this component. Another possible explanation is that R. H.'s hemoglobin may contain a greater percentage of sickle cell hemoglobin than B. H.'s. The difference in the slopes of the solubility curves of the hemoglobins from these two individuals is in large part due to low values of solubilities of B. H.'s hemoglobins at ionic strengths 4.7 and 5.0. Hemoglobin was present in but small excess in these experiments, and the same effect which diminishes the solubility of sickle cell trait hemoglobin when it is present in small excess accounts for these results.

Abnormally shaped erythrocytes are more susceptible to intravascular destruction than are normal erythrocytes.[24, 25] The great difference in the pathological consequences of sickle cell anemia and sickle cell trait as well as the gradations in the severity of different cases of sickle cell anemia can be related to the fraction of the total hemoglobin which is present as sickle cell hemoglobin.[1, 4, 10] When this fraction is sufficiently high as to cause sickling at the partial pressure of oxygen in venous blood, intravascular hemolysis and anemia result. The question may well arise as to why the combination of sickle cell hemoglobin and hemoglobin *d* causes anemia when hemoglobin *d* appears to be just as soluble as normal hemoglobin. One possible answer is that the percentage of hemoglobin *d* in the erythrocytes is low; another is suggested by the solubility data on sickle cell trait hemoglobin. As was previously pointed out, these data indicate the presence of an interaction between normal and sickle cell hemoglobin which stabilizes the solid phase and diminishes the solubility of a mixture of these two hemoglobins to less than a theoretically predicted value. A similar interaction may stabilize the aggregates in sickling erythrocytes; and if the stabilizing interaction between sickle cell hemoglobin and hemoglobin *d* were greater than that between sickle cell hemoglobin and normal hemoglobin, the presence of a high percentage of sickle cell hemoglobin need not be postulated in order to explain an increased susceptibility to sickling. The same possibilities may be considered with regard to the origin of the anemia which accompanies the presence of the combination of sickle cell hemoglobin and hemoglobin *c* in an individual; in this case it has been determined electrophoretically that the erythrocytes in the known cases contain about 50% of hemoglobin *c*.[5]

The genetic picture in this family appears to be analogous to the pictures presented by the families in which the sickle cell-hemoglobin *c* combination[5] and the sickle cell-thalassemia combination[3, 4] are present. In each

of these cases an erythrocyte abnormality has been detected in the non-sickling parent of an individual having a syndrome resembling sickle cell anemia. In discussing one of these cases Neel[3] has presented three possible genetic mechanisms for the transmission of the sickle cell and thalassemia characters. The decision as to the actual mechanism involved must await the accumulation of more data involving similar families and the examination of the descendants of individuals possessing the same hemoglobin constitution as R. H. and B. H. If the marriage of R. H. or B. H. to an individual having only normal hemoglobin results in a child having only normal hemoglobin, non-allelism of the genes responsible for the formation of sickle cell hemoglobin and hemoglobin *d* would be indicated. Although neither hemoglobin *c* nor hemoglobin *d* has been found free of other hemoglobins, this work does not provide any evidence that such states would necessarily be lethal.

The existence of other genetic forms of human hemoglobin is quite probable, but the problem of detecting and characterizing forms which do not produce hematologic and clinical disorders may prove difficult. Sickle cells have been observed in deer,[26] and an abnormal form of adult sheep hemoglobin has been detected in a lamb.[12] A systematic search would probably disclose the existence of chemically and genetically different forms of hemoglobin within other species. The search need not be confined to hemoglobins; the electrophoretically distinct components of crystalline serum albumin[27] and ovalbumin[28] may be of genetic significance.

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‡ Contribution No. 1609.

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*SPONTANEOUS AND ULTRA-VIOLET-INDUCED MUTATIONS TO PHAGE RESISTANCE IN ESCHERICHIA COLI**

BY VERNON BRYSON AND HARRIET DAVIDSON

BIOLOGICAL LABORATORY, COLD SPRING HARBOR, NEW YORK

Communicated by M. Demerec, October 27, 1951

One of the fundamental problems of genetics is the nature of differences between spontaneous and induced mutation. In higher organisms, mutations induced by ionizing radiations are known to include a relatively large number of chromosomal aberrations. Yet there is no doubt that spontaneously occurring chromosomal abnormalities have been important phylogenetically, and may themselves be the secondary consequences of spontaneous genetic changes.

Bonnier and Luning[1] observe that the regression line expressing a linear relationship between intermediate doses of x-ray and mutation frequency in Drosophila fails to intercept the point derived from untreated and slightly irradiated controls. Similar findings have been obtained by Spencer and Stern[2] with departures from linearity established as significant by Boag[3] using a probit diagram. It is at least possible that the deficit

of mutations observed following extrapolation of the regression line to control material is due to the inclusion, within any group of induced mutations, of types phenotypically similar but not necessarily identical to the entire potential array of spontaneous variants. An alternative interpretation would be that radiation, even at moderate dosage levels, accelerates unselectively the spontaneous mutation rate. This would lead to a more rapid production of mutants capable of spontaneous occurrence in the same relative proportions. No qualitatively new types would be anticipated, other than forms with spontaneous rates low enough to exclude detection in the majority of unirradiated populations.

Pending the development of an adequate system of microbial cytogenetics, comparisons of mutagenic effects on bacteria must depend on biochemical or morphological criteria, with no precise knowledge of the cytological consequences. Nevertheless, the known effect of x-rays on other biological material has prompted the choice of ultra-violet radiation in the present study as an agent with effects more likely analogous to spontaneous mutation. X-rays, nitrogen mustard and other chemical mutagens will be analyzed in supplementary investigations.

Most mutations occur at rates that are too low to afford quantitative information without considerable difficulty in obtaining samples of adequate size. Available data are therefore largely confined to the best studied genetic materials—maize[4, 5] and Drosophila.[6] The use of a screening technique allowing elimination of all non-mutant individuals automatically from the test population as employed in plant studies by Lewis[7] and in bacteriological investigation by Luria and Delbruck[8] and Demerec[9] offers the best solution to the problem of obtaining samples of sufficient numerical size to permit comparisons between spontaneous and induced mutation. A disadvantage of ordinary bacterial strains is the inability to localize precisely genetic differences as specific gene mutations on the linkage map. The recombining strain of *Escherichia coli* is not suitable for analysis of ultra-violet-induced mutations to phage resistance because of the lysogenic phage that it carries.[10] We have performed our comparison of ultra-violet-induced and spontaneous mutation with strain B/r of *E. coli*. This strain is resistant to radiation and penicillin,[11] nitrogen mustard,[12] oxidizing agents,[13] proflavine, crystal violet, potassium tellurite, and safranin. Most of these agents may be used selectively to obtain strain B/r from strain B.[12, 14]

The method employed has been to use bacteriophage T1 as the selective agent.[15] Mutations to T1 resistance in strain B/r are known to consist almost exclusively of two main types: B/r/1, resistant to T1 and sensitive to T5; and B/r/1, 5, resistant to both T1 and T5. The collective rate of mutation to T1 resistance is approximately 0.7×10^{-8} per cell per generation.[16] It is therefore possible by plating cell populations in excess of

this number on nutrient agar in the presence of phage to obtain considerable numbers of mutant colonies. All sensitive cells are lysed and provide no nutritional competition. By starting numerous independent cultures with small inocula of phage-sensitive cells, and isolating only a single mutant from one culture, it is possible to exclude the possibility that mutants will be related by descent from a mutant parent cell.

One hundred and sixty-four B/r/1 strains resistant to phage T1 and of independent spontaneous origin have been obtained. Each mutant was isolated by the following procedure. A stock broth culture of B/r, aerated during growth, was assayed for titer and found to contain a background not exceeding 200 B/r/1 per 10^8 cells when tested by three different T1 lysates. In eight separate experiments, a small initial population of phage-sensitive cells was obtained by diluting the stock culture and setting up independent subcultures containing broth volumes of 0.2 to 1 ml. and inocula of 2.5 cells to 1.2×10^3. The small independent cultures were incubated at 37° for two days. Following incubation, between 0.05 and 0.2 ml. of each subculture was spread on nutrient agar plates that had previously been covered with 2 to 5×10^8 lytic units of bacteriophage T1. After 48 hours of incubation, the isolated colony nearest the center of each plate was numbered, inoculated into a nutrient agar slant and streaked on a Petri plate containing nutrient agar. (Consecutive streaking serves to free the resistant strain from phage T1.) After incubation a single colony was therefore restreaked from the Petri plate. Part of a single colony from the second streaking procedure was later suspended in 2 ml. of broth, diluted 10^{-2} in saline and tested for phage resistance and for ability to grow on minimal media containing agar, water, glucose and inorganic salts. In determining the pattern of phage resistance the standard experimental series of seven phages (T1–T7) was reduced by the omission of T4 and T7, except in testing strains resistant to T3. Among spontaneous mutations, at least, testing with T3 is the practical antecedent of a test with T4 or T7, since one-step bacterial mutants sensitive to T3 have never been found to show resistance to T4 or T7.

Isolation of induced mutants for comparative purposes could have been performed with relative ease by irradiating bacteria, allowing them to grow through several divisions, and then selecting delayed mutants by the phage aerosol technique. The attendant disadvantages of using phage aerosol in a crowded laboratory caused us to employ an experimental method more closely related to the technique of isolating spontaneous mutants. By means of the same T1 lysates, 114 ultra-violet-induced mutants were isolated. The dose of ultra-violet energy, obtained from a GE T-15 mercury vapor lamp, was about 720 ergs per millimeter squared, sufficient in the various separate experiments to sterilize 90–99.6% of the treated cells. Bacteria were irradiated at a saline dilution of 10^{-1} in

Petri plates, shaken to minimize screening effects. The cells were then spread in 0.1-ml. quantities on nutrient agar plates and allowed to pass through from 2 to 9 divisions. The seeded plates were spread with from 2×10^8 to 10^9 lytic units of phage T1, incubated for 48 hours and a single mutant selected and purified in the same manner as described previously for spontaneous mutants.

Validity of Method Used in Isolating Induced Mutants.—Populations attaining a size equivalent to the reciprocal of the mutation rate will on the average contain one T1 resistant mutant. The wide statistical fluctuations in the random process of mutation make it possible that mutations will be found in rare instances within growing sensitive populations of relatively small size.[8, 17] There is always a possibility, therefore, that experiments designed for the isolation of induced mutants will include a

TABLE 1

COMPARISON OF T1-RESISTANT MUTANTS APPEARING ON NUTRIENT AGAR PLATES SPONTANEOUSLY, AND FOLLOWING ULTRA-VIOLET TREATMENT

NON-TREATED CELLS			ULTRA-VIOLET-TREATED CELLS		
TITER/PLATE	PROPORTION OF POSITIVE CULTURES	AVERAGE NUMBER MUTANTS PER POSITIVE CULTURE	TITER/PLATE	PROPORTION OF POSITIVE CULTURES	AVERAGE NUMBER MUTANTS PER POSITIVE CULTURE
3×10^3	0/2	...	1.5×10^3	0/5	...
6×10^4	0/6	...	5×10^4	1/5	4.0
1×10^5	0/2	...	1×10^5	1/2	1.5
5×10^5	0/6	...	5×10^5	10/10	63
2×10^6	1/6	1	1×10^6	10/10	15
3×10^6	2/6	1.5	4×10^6	27/27	342
7×10^6	0/7	...	6×10^6	30/30	208
2×10^7	0/2	...	1×10^7	10/10	313

few spontaneous mutants. The number of spontaneous mutants can be kept to a minimum by isolating only one mutant from any specific experimental population. Control experiments show the small probability that spontaneous T1-resistant mutants will arise in the absence of irradiation in sensitive bacterial populations at less than 10^7 viable cells (table 1).

The failure of treated suspensions to show a direct correlation between mean titer and mutant population is presumably due to the occasional presence of an early mutation which produced a clone of phage-resistant cells within a single colony during the period after ultra-violet treatment and plating, but before addition of phage and respreading of cells on the agar. When bacteriophage is spread over the agar following incubation of irradiated cells, individual clones are broken and distributed at random, contributing to a high variance in any analysis of the average number of mutants per plate. Table 1 shows that individual populations of less

than 2×10^7 viable cells are unlikely to contain mutants unless the cells have previously been irradiated. In isolating induced mutants, independent origin is assured by selecting from only one colony on a plate. A survey of all spontaneous and induced mutants describing resistance patterns to T1, T2, T3, T5 and T6, together with nutritional deficiencies, is given in table 2. Only strains resistant to T3 have been tested with T4 and T7.

Certain quantitative differences are at once apparent in comparing spontaneous and induced mutants. The proportion of mutants resistant to T1 but sensitive to T5 is 44% in the spontaneous group, and only 8% in the induced group. Two remarkable qualitative differences also appear. All mutants requiring a supplementary growth factor other than tryptophane are in the induced series. Within this group are found single mutants requiring thiamine, tryptophane + tyrosine + phenylalanine,

TABLE 2

PHAGE RESISTANCE PATTERN AND NUTRITIONAL REQUIREMENTS OF SPONTANEOUS AND ULTRA-VIOLET-INDUCED, T1-RESISTANT MUTANTS OF *E. coli*

	SPONTANEOUS				INDUCED			
			DEFICIENT				DEFICIENT	
	TOTAL	NON-DEFICIENT	TRYPTOPHANELESS	MISC.	TOTAL	NON-DEFICIENT	TRYPTOPHANELESS	MISC.
B/r/1	72	20	52	..	9	7	1	1[a]
B/r/1, 5	87	79	8	..	105	100	1	4[b]
B/r/1, 3, 4, 7	4	..	4	..	0	...	..	..
B/r/1, 3, 4, 7, 6	1	..	1	..	0	...	..	..
Total	164	99	65	..	114	107	2	5

[a] Methionineless.

[b] Thiamineless, aromaticless, purineless and serineless.

purines, serine and methionine. In contrast all patterns of phage resistance excluding the common patterns B/r/1 and B/r/1, 5 have arisen spontaneously. The comparative rarity of mutations leading to requirements for supplementary growth factors other than tryptophane, or to phage resistance other than B/r/1 and B/r/1, 5 types, makes a definite conclusion as to significance difficult. If distribution were truly random, the non-random occurrence of all the five rare phage mutants in one class, and the five aberrant deficient mutants (auxotrophs) in another would seldom occur on the basis of chance ($P = <0.002$).

Related to the low proportion of B/r/1 in the irradiated group is a comparatively small number of mutants requiring tryptophane. Of 72 spontaneous B/r/1, 52 required tryptophane. In the induced series only 1 of 9 required this amino acid. Requirement for tryptophane is less likely to occur in the mutation B/r → B/r/1, 5 than in B/r → B/r/1.

In the former class (B/r/1, 5) only 8 of 87 spontaneous mutants required tryptophane, and only 1 of 105 induced B/r/1, 5 had a tryptophane requirement. The greater proportion of tryptophaneless in the spontaneous population is probably a significant departure from random distribution. Some investigators regard the appearance of tryptophane requiring B/r/1, 5 mutants as evidence of a two-step process: B/r/1 *tryp*$^-$—B/r/1 *tryp*$^-$/1, 5. In the second step, sensitivity to T1h host range mutants in T1 phage would simultaneously be lost. Although known as an experimental possibility, there is no evidence that two-step origin is required. The deficit of B/r/1 mutants in the induced class (table 2) is not due to differential elimination of these individuals by host range phage mutants (T1h) in the lysates employed for isolation, since the same lysates were used in obtaining virus-resistant bacteria of both spontaneous and induced origin.

Another difference in the spontaneous and induced populations arises in the proportion of mutants that show partial resistance to phage T1. Such partial resistance is exhibited by 26 of the induced B/r/1, 5 mutants, and by one of 3 induced B/r/1 mutants, including a methioneless strain. By comparison only 3 strains partially resistant to T1 were found in the entire group of 164 spontaneous mutants. Partially resistant strains are called $B/1_p$, $B/1_p5_p$, $B/1_p5$, etc.

Partial resistance is characterized by reduced growth rate in broth containing over 5×10^9 lytic units per milliliter, but normal growth rate if the phage concentration is reduced to about 10^8 lytic units per milliliter. In phage concentrations of 10^8 per cubic centimeter, the composition of the $B/1_p$ culture remains unchanged, but in phage concentrations over 5×10^9 a culture grown from a small inoculum contains primarily B/r/1. A similar effect of phage concentration is observed on nutrient agar, where colonies of $B/r/1_p$ do not grow to normal size with 24 hours' incubation at 37°C. if high concentrations of T1 are present. Colonies isolated from heavily phaged plates are B/r/1.

Dilution assays of $B/1_p$ cultures were made on unphaged plates, and on plates with sufficient phage to delay the appearance of visible colonies by about 10 hours. The phaged plates contained more than enough T1 to eliminate all fully sensitive B/r bacteria before B/r/1 mutations were likely to occur. The number of cells initiating colonies on phaged and unphaged plates was most frequently equal, showing that the non-B/r/1 cells in the $B/r/1_p$ culture cannot be B/r. From these facts we conclude that the predominating type of cell in the $B/1_p$ culture is in itself responsible for many of the peculiarities of the culture, and that this cell is different from B/r and B/r/1.

Many strains with partial resistance produce plaques when exposed to concentrations of phage that would give confluent lysis of a sensitive

strain. Thus nutrient agar plates spread with three independent B/r/1_p strains and 3×10^3 lytic units of T1 gave 10, 38 and 107 cloudy plaques respectively. At high phage concentrations the cloudy plaques become confluent and large clear plaques appear on two specific B/r/1_p strains as a type of host range variant capable of completely lysing the partially resistant bacteria. All B/r/1_p strains were tested for efficiency of plating. This expresses the ratio of cloudy plaques found on B/r/1_p to clear plaques observed on the parent strain, B/r. Where plaques were found on B/r/1_p the correlated low efficiency of plating was not due to poor adsorption of phage in a test of one strain.

Exposure of irradiated bacteria to mixtures of phages is a more efficient method of detecting multiple patterns of phage resistance and may reveal complex types as noted in the spontaneous group of table 2. Occurrence of unique auxotrophs in the treated sample is thought to be of greater significance, particularly since other investigators with considerable experience in the nutritional requirements of spontaneously arising phage-resistant bacteria have found no auxotrophs that did not require tryptophane[18] or a known biochemical precursor. Anderson obtained 27 independent strains requiring tryptophane and resistant to T1. One of these also had a deficiency for proline.

Tryptophane-requiring mutants not resistant to T1 may easily be obtained by the penicillin method.[19] The association of resistance to T1 and tryptophane deficiency can nevertheless occur as the result of a single mutation or complex of mutations producing the related phenotypic changes either at once or in sequence too rapid to be detected by our present methods. Resistance to T1 and T5, or to T3, T4 and T7 also is associated.[15] Anderson has approached this problem by assuming that a series of catenary reactions exists, governed by specific enzymes and leading to phage sensitivity. The dichotomous nature of these processes implies that genetic blocks occurring early in a series of branching events would influence ability of the cell to act as host for several viruses, leading to familiar mutants such as B/1, 5 or B/3, 4, 7. Inability of the cell to synthesize tryptophane interferes with T1 reproduction, and also prevents cell multiplication on minimal media.

By Anderson's interpretation, reverse mutation from a mutational block interfering with a common precursor for phage reproduction and cell growth on minimal media should restore the original wild-type phenotype. Tests to be reported show that in specific instances it is possible for nutritionally deficient, phage-resistant strains to yield prototrophs without loss of the resistance pattern to phage. If we adopt the scheme of Anderson, a loss of nutritional deficiency without change of phage resistance pattern could be interpreted, not as a reverse mutation affecting the site of the original enzymatic block, but as an additional mutation influencing another position

in the chain of biochemical events and restoring prototrophy (non-deficiency) without relation to phage sensitivity. It would then be probable that the "reverted" types obtained from ultra-violet-induced phage-resistant auxotrophs would differ genetically from spontaneous prototrophs of the same resistance pattern. Differences between parental strains and prototrophs arising by reverse mutation are often observed.[20] The Anderson hypothesis has served a useful purpose, but becomes increasingly forced as new varieties of T1-resistant mutants are found.[21]

Auxotrophic strains requiring growth supplements other than tryptophane, or strains resistant to more than one complex of phages (e.g., B/r/1, 3, 4, 7, 6, table 2) occur far more frequently than expected on the basis of simultaneous origin of independent mutations in a single individual.[15] In explanation it could be assumed that mutation causing one class of phenotypic changes greatly increases the probability of a simultaneous or rapidly ensuing mutation of another type capable of independepe occurrence at a lower rate. Another interpretation is that the phenotynt $a + b + c$ is not due to the simultaneous genetic events $a' + b' + c'$, but arises from the independent mutation d'. The ultimate solution of these problems will not alter the fact that in this experiment induced mutations differ from spontaneous.

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EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. X. REPRODUCTIVE ISOLATION BETWEEN DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS UNDER NATURAL AND UNDER LABORATORY CONDITIONS

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The Problem.—The species *Drosophila pseudoobscura* and *Drosophila persimilis* are reproductively isolated. The isolation is the outcome of cumulative action of several isolating mechanisms, none of which is by itself fully effective. The distribution regions of these species are different, but they overlap broadly. Within the zone of the overlap, *D. pseudoobscura* is relatively more common in warmer and drier habitats than *D. persimilis*, but they occur together in many localities. Sexual isolation can be demonstrated, in laboratory experiments, by placing females of both species with males of one of them. Homogamic matings (within the species) are usually more frequent than heterogamic ones (between species).[1–4] Sexual isolation is also found when females and males of both species are present together in the same container.[5] The intensity of the isolation is modified by environmental[2, 4, 6] as well as by genetic variables,[5] but in no experiment has a complete isolation been found. The interspecific matings result, under laboratory conditions, in production of hybrids which appear to be fully as vigorous as the parents. The F_1 males are, however, sterile, and the offspring of the F_1 hybrid females show a pronounced constitutional weakness.

In nature, not a single interspecific hybrid has ever been encountered, although certainly hundreds, and possibly as many as two thousand, flies collected in localities where both species occur together have been subjected at various times to cytological or genetic analysis which would detect such hybrids. In view of the incompleteness of the sexual isolation observed in laboratory experiments this absence of natural hybridization is startling. For direct observation in laboratory cultures shows that males court females of either species indiscriminately. Females accept conspecific males more readily than foreign ones. Experimental data are consistent with the hypothesis that the frequency of homogamic and heterogamic matings is a function of the number of encounters between flies of opposite sex, the probability of copulation taking place being greater in conspecific than in heterospecific encounters.[3, 7, 8] The presence of an excess of potential conspecific mates does not, in experiments, preclude the occurrence of heterogamic matings. How can the absence of hybrids in nature be reconciled with the incompleteness of the sexual isolation

found in laboratory experiments? One may, perhaps, conjecture that the hybrids are poorly viable under natural conditions. There is no evidence to support this supposition. The parental species may be segregated in different microenvironments within the same general habitat, and no opportunity may arise for their meeting (ecological isolation). Finally, the sexual isolation may be much stronger in nature than under laboratory conditions.

Ecological Isolation.—Drosophila flies show a diurnal cycle of activity in their natural habitats. If bait is exposed to attract the flies, they visit the bait chiefly for a few hours following the sunrise and preceding the sunset. No flies at all come during the night, and ordinarily very few come during the noon hours.[9]

In the summer of 1951, banana bait fermented with Fleischmann's yeast was exposed in the vicinity of the Carnegie Institution Garden, near Mather, Calif. Some of the females captured on this bait were allowed to breed in individual culture bottles. When fully grown larvae appeared in the cultures, some salivary gland smears were stained in acetic orceine. Inspection of the chromosomes in such smears makes it possible to distinguish the offspring of *D. pseudoobscura* and *D. persimilis* mothers.[9] It soon became apparent that the proportion of *D. persimilis* is higher among the flies collected during the morning periods of activity. In all, 355 females from evening catches were analyzed, and 29, or 8.2% of them, proved to be *D. persimilis*. Among the 128 flies from the morning catches, 33, or 25.8%, were *D. persimilis*. The difference is statistically quite significant (chi-square 26.23). It follows that, at least under mid-summer conditions in the Transition Life Zone of the Sierra Nevada of California, *D. persimilis* is relatively more active in procuring food in the morning and *D. pseudoobscura* in the evening. Since the number of encounters between flies of the opposite sex is increased by their wanderings in the search for food, this difference in the activity rhythms of the two species undoubtedly contributes toward their reproductive isolation. But this isolation is far from complete.

D. pseudoobscura and *D. persimilis* have somewhat different food preferences. This has been shown by exposing as bait several different species of yeast and recording the proportions of the two species attracted to such baits.[10] That this isolating agent is relatively weak has, however, been demonstrated by Carson,[11] who ascertained that adults of both species feed on, and larvae develop in, the same slime fluxes on certain oak trees.[12]

Sexual Isolation under Natural Conditions.—To test the possibility that sexual isolation is more nearly complete in nature than under laboratory conditions, copulating pairs of flies were collected at Mather, Calif., between June 26 and August 16, 1951. Cups with fermenting banana were exposed and were inspected about three times per hour during the morning

or the evening periods of the fly activity (see above). The flies found in the cups in copula were isolated and placed in separate culture bottles. Inspection of the chromosomes in the salivary glands in their offspring made it possible to distinguish the matings in which both parents were *D. pseudoobscura*, or both *D. persimilis*, or in which the parents belonged to different species. The species of the surviving male parent was in some cases also checked by the method of Rizki.[13] These checks agreed with the results of the chromosome investigation.

In the summer of 1951, *D. persimilis* was relatively much less common among the flies in the Mather locality than *D. pseudoobscura*. As indicated above, only about 8% of the *obscura*-like flies caught during the evening periods of activity, and about 26% of the flies caught during the morning were *D. persimilis*, the remainder being *D. pseudoobscura*. Among the 305 copulating pairs collected in the evenings, 280 matings involved *D. pseudoobscura* females and males, and 25 matings involved *D. persimilis* females and males. None represented interspecific matings. Among the 90 pairs copulating in the morning, 65 consisted of two *D. pseudoobscura* and 25 of two *D. persimilis* partners. Again, no interspecific matings were observed.

All of the 395 copulae observed under conditions as closely approaching natural ones as was possible to obtain represented homogamic matings. Since the two parental species were not equally frequent in the environment, it is particularly significant that 50 copulating pairs in which both parents were *D. persimilis* were obtained. In the Mather locality during the summer of 1951, *D. persimilis* flies encountered individuals of the opposite sex of *D. pseudoobscura* more frequently than they did potential mates of their own species. Nevertheless, no heterogamic matings were observed.

Crossing Laboratory-Bred and Wild Flies.—The observations described above demonstrate that sexual isolation between *D. pseudoobscura* and *D. persimilis* is far stronger under natural than under laboratory conditions. In nature, the isolation seems to be, in fact, absolute. An attempt has been made to find out whether this difference is due to the behavior of the females or of the males, or of both sexes.

Groups of two *D. pseudoobscura* and two *D. persimilis* females reared in the laboratory were introduced together with four males collected in nature into glass vials which contained a drop of yeasted banana as food. The species of the females was distinguished in some experiments by employing a *D. persimilis* strain homozygous for the recessive gene orange (an eye color mutant). In other experiments, wild-type strains of both species were used, but females of one of the species had a part of the right wing, and in the other species part of the left wing cut off. The females were made receptive by aging them without males for about ten days. The

males were collected at Aspen Valley, several miles distant from Mather, where the proportion of *D. persimilis* was appreciably higher than in the latter locality. The vials were placed on white paper, and the behavior of the flies was watched. As soon as a copulating pair appeared in one of the vials, the pair was isolated, the male was killed, and its genitalia were examined as indicated by Rizki[13] to determine its species. Among the 34 pairs so examined the following combinations were found:

D. pseudoobscura ♀	*D. pseudoobscura* ♀	*D. persimilis* ♀	*D. persimilis* ♀
D. pseudoobscura ♂	*D. persimilis* ♂	*D. pseudoobscura* ♂	*D. persimilis* ♂
20	2	2	12

It is evident that, although homogamic matings are distinctly preferred, the nature-grown males do not show, at least when confined in vials, an aversion to mating with females of foreign species. The discrimination is probably exercized by the females. Experiments designed to test this by exposing females collected in nature to laboratory-grown males have, however, failed. Most of the females found in natural habitats are already inseminated and accept males with difficulty. Furthermore, it would be desirable for such experiments to have a population in which females of *D. pseudoobscura* and *D. persimilis* would be about equally frequent, and no such population was located in the vicinity of Mather during the summer of 1951.

Conclusions.—The gene exchange between the natural populations of *Drosophila pseudoobscura* and *Drosophila persimilis* is prevented by cooperation of at least seven reproductive isolating mechanisms, as well as by partial geographic isolation. The reproductive isolating mechanisms are as follows: (1) *D. persimilis* occur more often in cooler and moister habitats than *D. pseudoobscura*. (2) The food preferences of the two species are somewhat different. (3) *D. pseudoobscura* is relatively more active in the evening and *D. persimilis* in the morning. (4) Females of both species accept males of their own species more readily than they do heterospecific males. (5) The interspecific copulations result in transfer of less sperm than the intraspecific ones.[14] (6) The F_1 hybrid males are sterile. (7) A breakdown of the viability is observed in the backcross progenies.

The above isolating mechanisms are by no means equivalent in effectiveness. It appears that the absence of interspecific hybrids in natural habitats is due chiefly, if not entirely, to the sexual isolation. Yet, the sexual isolation is, as far as known, never complete in laboratory experiments. The cause of this difference in the behavior of the flies in nature and in the laboratory is unknown.

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*THE INDUCTION OF ACTIVATED, STABLE STATES IN THE CHROMOSOMES OF TRADESCANTIA BY INFRA-RED AND X-RAYS**

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Infra-red by itself is ineffective in inducing chromosomal aberrations. When employed in combination with x-rays, however, it is an effective potentiating agent, and significant increases in the frequency of aberrations are readily obtained. This phenomenon was first demonstrated in Drosophila by Kaufmann, Hollaender, and Gay.[1] They succeeded in showing that an exposure of Drosophila males to infra-red prior to x-radiation increased the frequency of aberrations for any given dose of x-rays, but that a post-treatment was generally ineffective except under certain circumstances.[2] Subsequent studies in Tradescantia have revealed that

infra-red is an effective potentiating agent when used either as a pre- or as a post-treatment, although not equally so for all types of aberrations.[3-4] In addition, the duration of time between exposure of the inflorescences to infra-red and to x-rays was shown to be unimportant; the effect, once induced, was relatively permanent and did not decay with time to any appreciable degree or in any consistent manner.

Two hypotheses have been advanced to account for the action of infra-red in these combined radiation experiments. The Drosophila workers have suggested that infra-red acts principally to favor the process of recombination of broken ends over that of restitution.[1, 6] According to this hypothesis, x-rays alone can induce breaks; infra-red simply influences their subsequent behavior. We have assumed, on the other hand, that the primary action of infra-red is such as to increase the frequency of primary breaks which may or may not become involved in the process of recombination.[3-5] As a corollary hypothesis, we are now proposing that both infra-red and x-rays can induce activated states in the chromosome which, under the influence of the other type of radiation, are more readily moved along the reaction pathway which leads to complete breakage of the chromosomes. Following the ideas of Eyring and Stearn[7] (see also McElroy and Swanson[8]), it is considered that the activated states occupy a stable position in an energy depression somewhere along the reaction pathway, and that their subsequent behavior will depend upon the environmental factors which impinge upon them. The activated states are different for each type of radiation, and our studies indicate that each is acted upon by the other type of radiation, i.e., the radiations must be in combination for an increase in breaks to be achieved. Temperature shocks were therefore employed to test for the presence of these activated states since many studies of both a biological and a physical nature have indicated that such states are sensitive to such environmental changes.

Experimental Procedures and Results.—Methods for the exposure of inflorescences of Tradescantia to infra-red radiation have been described previously.[3, 5] The infra-red source used in these experiments was a 250-watt drying lamp, the exposure time was three hours, and the radiation was passed through a Corning No. 2540 glass filter. All x-ray exposures were made at 80 kv. and 10 ma., with the total dose in each case being 67 *r* units delivered in one minute. All temperature shocks consisted of immersion of the inflorescences in water at 48°C. for 30 seconds. This temperature was found to be effective in bringing about a change in the state of sensitivity of the chromosomes, and was therefore adhered to in all of the experiments herein reported. The chromosomes were smeared 22–24 hours after x-radiation regardless of what other treatments were given. Only metaphase figures were scored.

Table 1 summarizes the data obtained from two experiments in which

a temperature shock was interposed between the infra-red and x-ray exposures. The *x-ray* and *temp.-x-ray* series serve as controls against which the *infra-red-temp.-x-ray* and the *infra-red-x-ray* series can be evaluated. Whenever a temperature shock was employed it preceded the x-ray exposure by one hour to avoid any effects which temperature might have on the x-ray effects *per se*. A comparison of the *x-ray* and the *temp.-x-ray* series indicates that there is no such effect. All categories of aberrations in the *infra-red-x-ray* series are significantly increased over those in the *x-ray* and *temp.-x-ray* series, while the data in the *infra-red-temp.-x-ray* show no significant increases. A temperature shock of the magnitude used clearly removes the effect induced by the infra-red, and the chromosomes thus treated react to x-rays as if no prior infra-red exposure had been made. The action of infra-red on chromosomes therefore appears to be such as to raise them to a state of higher reactivity to subsequent doses of x-rays, a state from which they can be returned to one of normal reactivity by the temperature shock.

TABLE 1

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN BETWEEN EXPOSURES OF INFRA-RED AND X-RAYS. ALL *P* VALUES DERIVED FROM χ^2 CALCULATIONS, AND IN TERMS OF THE *X-Ray Control* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	1800	4.72	6.34	1.39
Temp.-x-rays	2400	4.55	6.43	1.29
		P = 0.70-0.80	0.80-0.90	0.70-0.80
Infra-red-temp.-x-rays	2580	4.31	7.06	1.32
		P = 0.50-0.70	0.20-0.30	0.80-0.90
Infra-red-x-rays	1800	8.55	9.62	3.22
		P = <0.001	<0.001	<0.001

It was then decided to test the effect of a similar temperature shock when applied between x-rays and infra-red, the latter being given as a post-treatment. It was first necessary, however, to make certain that a temperature shock of this magnitude would not interfere with the capacity of the chromosome to respond to the influence of infra-red. The data in table 2 were obtained with this in mind, and it is evident that the *infra-red-x-ray* and the *temp.-infra-red-x-ray* series are similar for all categories of chromatid aberrations.

Having established this fact, the data in table 3 can be more properly evaluated. The temperature shock in this experiment was delayed for an hour after the end of the x-ray exposure to avoid any possible interference with the processes leading to recombination. In the *x-ray-infra-red* series, the lack of increase in the isochromatid deletions agrees with the data from earlier experiments[3] (Swanson's[4] later conclusions bearing on this subject are incorrect, and are to be disregarded). The chromatid deletions

and the exchange aberrations are increased as expected. When the temperature shock is interposed between the two radiations, the isochromatid deletions remain unaffected, the chromatid deletions are significantly decreased, and the exchanges are returned to a level comparable to that induced by x-rays alone. It is reasonable to conclude, as a consequence, that the temperature shock acts not to decrease the capacity of the chromosome to react to infra-red, but rather to "desensitize" the chromosomes by eliminating the x-ray induced activated states which ordinarily respond to the influence of infra-red.

Discussion.—The experiments just cited indicate that both x-rays and infra-red can induce altered states in the chromosome which have no morphological expression, but which respond in combined radiation experiments in such a manner as to increase significantly the frequency of aberrations. These states can persist unchanged for long periods of time

TABLE 2

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN PRIOR TO INFRA-RED, WITH INFRA-RED BEING USED AS A PRETREATMENT. *P* VALUES ARE DERIVED FROM χ^2 CALCULATIONS WITH P^x BEING COMPUTED IN TERMS OF THE *X-Ray Control* SERIES, AND P^i IN TERMS OF THE *Infra-Red-X-Ray* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	3616	5.25	6.75	1.41
Temp.-x-ray	3600	5.75	7.25	1.42
		P^x = 0.70–0.80	0.30–0.50	0.90–0.95
Infra-red-x-rays	3600	8.84	8.35	3.00
		P^x = <0.001	0.01–0.02	<0.001
Temp.-infra-red-x-ray	3864	8.41	8.87	2.82
		P^x = <0.001	0.01–0.001	<0.001
		P^i = 0.50–0.70	0.50–0.70	0.50–0.70

at room temperatures without an appreciable decay in the induced effect; they can, on the other hand, be removed from participation in the breakage-recombination processes by a temperature shock. Additional, but incomplete, data also show that an infra-red effect cannot be induced if the inflorescences are irradiated at temperatures of 23°C. and above (this, despite the fact that if the infra-red is given at a lower temperature the states are nevertheless stable when the inflorescences return slowly to room temperature), but that at 18°C. and lower the infra-red effect is quite pronounced. There is also the suggestion in these data that temperatures lower than 18°C. during infra-red exposure—e.g., 12° and 4°C.—led to progressively greater increases. The exact temperature relationships remain to be worked out in detail, but the available data are in agreement with expectations if it is assumed, as is likely, that the lower the temperature the greater would be the number of activated states retained, and the longer would be their half-life.

These considerations suggest that we are dealing with induced states of a higher energy content than that characteristic of the unirradiated molecular structure of the chromosome, and that in this energized condition the chromosomes react with greater fragility to subsequent radiation. If this be the case, then the further assumption must be made that the activated states induced by infra-red and by x-rays are dissimilar either in the energy levels attained or in the reaction pathways subsequently followed. This assumption is made necessary by the fact that while infra-red, when employed in post-treatment exposures, can raise the x-ray induced states to the level of primary breaks and thus increase the frequency of aberrations, additional infra-red radiation cannot do likewise with those states previously induced by itself. That is, infra-red cannot by itself induce chromosomal aberrations regardless of the duration of exposure or, so far as our experiments show, of the conditions of exposure.

TABLE 3

EFFECT OF A TEMPERATURE SHOCK WHEN GIVEN BETWEEN EXPOSURES TO X-RAYS AND INFRA-RED. P VALUES ARE DERIVED FROM χ^2 CALCULATIONS WITH P^x BEING COMPUTED IN TERMS OF THE *X-Ray Control* SERIES, AND P^i IN TERMS OF THE *X-Ray-Temp.-Infra-Red* SERIES. DATA IN PER CENT OF ABERRATIONS ON A CHROMOSOME BASIS

TREATMENT	NUMBER OF CHROMOSOMES	SINGLE DELETIONS	ISOCHROMATID DELETIONS	EXCHANGES
X-ray control	6300	4.27	4.49	1.43
X-ray-temp.	3900	4.60	4.00	1.46
		P^x = 0.30–0.50	0.20–0.30	0.80–0.90
X-ray-temp.-infra-red	4800	4.79	4.90	1.37
		P^x = 0.10–0.20	0.20–0.30	0.80–0.90
X-ray-infra-red	6000	5.35	4.08	3.72
		P^x = 0.01–0.001	0.20–0.30	<0.001
		P^i = 0.10–0.20	0.05–0.02	<0.001

If we have correctly interpreted the present data, they provide the first concrete evidence that radiations can induce activated, stable states in the genetic material of the cell. The nature and frequency of these states is not known, and it is unlikely that the present technique, which depends upon the determination of an end-product rather than an exact and quantitative description of the states themselves, will provide other than preliminary data relating to their presence or absence, but an understanding of them is essential to an understanding of the actions of radiation as they affect the heriditary potential of the cell. Examples from other fields, however, can provide us with possible clues and avenues of approach to a further study of these effects. Phosphorescent materials, for example, exhibit a strikingly parallel behavior to the conditions herein described. Metastable states can be induced photochemically, they can persist for considerable intervals of time, and they can be returned to a ground state by high temperatures.[9] An analogous situation also holds for certain

of the high polymers of a synthetic nature.[10] Both of these examples may be explained by assuming that energy in the form of photoelectrons is trapped within the irregularities of a lattice network; in the case of phosphorescent materials, impurities in the crystal structure are involved in creating distortions which facilitate the trapping process. The situation found in high polymers, however, is likely to be more closely analogous to that described here not only because of the polymeric nature of the chromosome, but because of the roles in polymerization processes played by peroxides and oxygen. The importance of both in the interpretation of radiation effects on genes and chromosomes has been discussed by many authors, and need not be considered here.

Final mention should be made of the fact that mustard, which in many respects acts in a manner similar to x-rays, can apparently induce activated genic states which are temperature sensitive.[11] It has also been shown that the mustard effects on chromosome breakage can be considerably enhanced by infra-red radiation.[12] It is possible, in the light of these studies, that a reinterpretation of radiation effects on genetic material in terms of activated states may assist in an elucidation of the role played by such environmental agents as oxygen tension and temperature. In a theoretical way, this topic has been considered by McElroy and Swanson,[8] but further experimentation is clearly needed.

Summary.—A temperature shock of 48°C. for 30 seconds, given between exposures of inflorescences of *Tradescantia* to infra-red and x-rays, and to x-rays and infra-red, effectively prevents the potentiating action of infra-red from being expressed in the form of an increase in the frequency of chromosomal aberrations. It is proposed that both infra-red and x-rays induce activated, stable states in the chromosomes, and that in combined radiation experiments these states are raised to the level of primary breaks which may remain open as deletions or enter into recombination with other similarly broken ends to give gross aberrations. These states are removed by high temperatures when once induced, and have a transitory, and hence undetectable, existence if induced at temperatures of 23°C. and above.

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THE INTERACTION OF A MINUTE MUTATION WITH A SEX-LINKED LETHAL OF DROSOPHILA MELANOGASTER

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Despite the fact that the analyses of lethal mutations of Drosophila present a promising approach to the study of gene action, phase specificity has been determined for only a few and the possibility of interaction between lethals and other mutants has received little attention.[1] An experiment was designed, therefore, to determine the effect of a minute mutation, *M*(3)*w*, upon the lethal, *l*(1)7, and the average time of death and the distribution of deaths among the *l*(1)7 larvae.

Method.—The recessive sex-linked lethal *l*(1)7, characterized by the appearance of melanomas, was most recently investigated by Russell[2] who estimated that death occurs in the male larvae about 100 hrs. after hatching. The minute *M*(3)*w*, is lethal when homozygous, but Dunn and Mossige[3] found that the heterozygous mutation causes a pronounced retardation in the rate of larval development. To test the effect of this retardation upon the activity of *l*(1)7 it was necessary to obtain flies which could be crossed to produce male larvae containing *l*(1)7 only and other males with both *M*(3)*w* and *l*(1)7. Accordingly, two stocks were prepared one of which contained $\frac{e \quad l(1)7}{e \ \text{In(1)}dl\text{-}49}$ females and the other $\frac{M(3)w \quad +}{\text{In(3R)C} \quad e}$ males. In(1)*dl*-49, in addition to carrying the marker gene yellow, served as a crossover suppressor in the *l*(1)7 region of the sex chromosome while In(3R)C performed a similar function in the *M*(3)*w* and ebony region of the third chromosome. Crossing these two types produces an F_1 generation containing larvae of eight different genotypes among which are $\frac{M(3)w \quad +}{e}$ *l*(1)7 males and $\frac{\text{In(3R)C} \quad e}{e}$ *l*(1)7 males. Several hundred eggs from such a cross were collected and the hatching larvae removed at hourly intervals and placed in Stender dishes containing yeasted Cream-of-Wheat medium. Between 40 and 50 hrs. after hatching,

the larvae were examined and 102 $\frac{\text{In(3R)C}}{e}\ \frac{e}{e}\ l(1)7$ males and 25 $\frac{M(3)w}{e}\ \frac{+}{e}\ l(1)7$ males were separated from the rest. The male larvae were distinguished from the females by comparing the gonad size and the $l(1)7$ males were separated from the In(1)*dl*-49 males by the color of the mouth parts. Since the $l(1)7$ males containing $M(3)w$ were heterozygous for ebony and the $l(1)7$ males without $M(3)w$ were homozygous for this gene, the two types could be distinguished and separated by an examination of the spiracle sheath pigmentation. Thereafter the larvae were examined at 2-hr. intervals and the time of death was recorded for each. To determine what effect if any, the In(3R)C has upon the $l(1)7$, 46 $\frac{e}{e}\ l(1)7$ male larvae obtained from the $l(1)7$ stock were similarly timed for comparison with the $\frac{\text{In(3R)C}}{e}\ \frac{e}{e}\ l(1)7$ type. Overcrowding was carefully avoided and all eggs and larvae were maintained at 25°C.

TABLE 1

AVERAGE AGE AT DEATH ATTAINED BY $l(1)7$ MALE LARVAE OF THE THREE DIFFERENT GENOTYPES

TYPE NO.	LARVAL GENOTYPE	NUMBER OF INDIVIDUALS	AVERAGE AGE AT DEATH IN HOURS: MEAN ± S.E.	S.D.	TEST OF SIGNIFICANCE
1	$\frac{e}{e}\ l(1)7$	46	102.5 ± 1.47	9.94	Between 1 and 2
2	$\frac{\text{In(3R)C}}{e}\ \frac{e}{e}\ l(1)7$	102	107.5 ± 1.28	12.95	$t = 2.56$ $P = 0.01$
3	$\frac{M(3)w}{e}\ \frac{+}{e}\ l(1)7$	25	126.9 ± 8.12	40.60	Between 2 and 3 $t = 2.37$ $P = 0.02$

Results and Discussion.—The results are given in table 1. A small significant difference of 5 hrs. exists between types 1 and 2 but this may be due to some effect of In(3R)C or to a difference in residual heredity since the two types are not isogenic. A much larger difference of 19.4 hrs. exists between 2 and 3. Here the difference cannot be ascribed to a difference in residual heredity because the two types occur as sibs and are presumably isogenic. A retardation effect of this magnitude has never been associated with the ebony allele when homozygous, so that the only remaining genetic difference between types 2 and 3 larvae is the substitution of $M(3)w$ for In(3R)C. Since the effect, if any, of the inversion is apparently quite small, the difference of 19.4 hrs. in the average at death can be safely attributed to the retarding action of $M(3)w$. Dunn

and Mossige[3] and Brehme[4] found that the delaying action of heterozygous $M(3)w$ occurred during larval life probably before the third instar. Since these results indicate that $M(3)w$ exerts some effect upon $l(1)7$ larvae, it follows that the retarding effect of $M(3)w$ must be initiated during the first instar because $l(1)7$ larvae ordinarily die before passing this stage. Furthermore, the large standard deviation of type 3 may be an indication that the time at which the minute begins to act is highly variable. It would be interesting to investigate by this method the characteristics of the phase specificity of other minute mutations.

Hadorn[1] points out that further insight into the action of a lethal gene can be gained by determining the distribution of the age at death in larvae carrying this gene. Accordingly such an analysis was performed where sufficient data were available, in this case upon the 102 $l(1)7$ individuals of type 2. The results of the analysis indicate a normal distribution which offers some confirmation of Russell's[2] view that death of $l(1)7$ larvae is due to gut closure and subsequent starvation.

Summary.—The presence of the $M(3)w$ mutation in larvae hemizygous for $l(1)7$ is found to delay the time of death by about 20 hrs. Some conclusions concerning the phase specificity and action of $M(3)w$ and $l(1)7$ are presented.

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THETA RELATIONS WITH SPHERICAL HARMONICS

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This paper is presented separately because of the arithmetical connotations implicit, but it has been detached from another paper in which larger classes of Fourier series and integrals are to be analyzed.

H. Hecke[1] and A. Schoeneberg[2] have found that the classical relation

$$\sum_{(n)} e^{-\pi t(n_1^2 + \ldots + n_k^2)} = t^{-(2/k)} \sum_{(n)} e^{-(\pi/t)(n_1^2 + \ldots + n_k^2)} \qquad (1)$$

may be extended to

$$\sum_{(n)} P_g(n_1, \ldots, n_k) e^{-\pi t(n_1^2 + \ldots + n_k^2)} = i^g t^{-(k/2)-g} \sum_{(n)} P_g(n_1, \ldots, n_k) e^{-(\pi/t)(n_1^2 + \ldots + n_k^2)},$$

where

$$P_g(x_1, \ldots, x_k) \tag{2}$$

is any homogeneous polynomial of degree g which is a solution of the Laplacean $\Delta P = 0$ in E_k. The most comprehensive version of (1) is

$$\sum_{(n)} e^{-\pi t Q(n+\alpha) + 2\pi i \Sigma_j (n_j + \alpha_j)\beta_j} = t^{-(k/2)} (\det Q)^{-1/2} \sum_{(n)} e^{-(\pi/t)Q'(n+\beta) - 2\pi i \Sigma_j n_j \alpha_j}, \tag{3}$$

where $Q(t) = Q(t_1, \ldots, t_k)$ is an arbitrary real positive definite quadratic form, and $Q'(t)$ is its "inverse," and it has been extended in a similar fashion as follows.

THEOREM 1. *We have the relation*

$$\sum R_g(n+\alpha) e^{-\pi t Q(n+\alpha) + 2\pi i \Sigma_j (n_j + \alpha_j)\beta_j} = i^g t^{-(k/2)-g} (\det Q)^{-1/2} \sum R_g'(n+\beta) e^{-(\pi/t)Q'(n+\beta) - 2\pi i \Sigma_j n_j \alpha_j}, \tag{4}$$

where $R_g(t)$ arises from a harmonic polynomial (2) by a substitution $x = At$ which transforms $\sum_j n_j^2$ into $Q(n)$, and $R_g'(t)$ arises by the inverse reciprocal substitution $x = (A^*)^{-1}t$.

This was proved by deriving relation (3) with respect to the β's, in accordance with Maxwell's description of spherical harmonics, but the following different procedure has its merits. It suffices to prove

$$\int_{E_k} P_g(x_j) e^{-\pi(x_1^2 + \ldots + x_k^2) + 2\pi i \Sigma_j x_j y_j} dv_x = i^g P_g(y_j) e^{-\pi(y_1^2 + \ldots + y_k^2)},$$

since (4) will then follow from Poisson's formula after performing a suitable affine transformation first;[3] and this in its turn follows immediately, by way of the relation

$$\int_0^\infty e^{-p^2 t^2} J_\nu(t)^{\nu+1}\, dt = \frac{1}{(2p^2)^{\nu+1}} \exp\left(-\frac{1}{4p^2}\right),$$

from the following theorem which we will prove.

THEOREM 2. *If $\varphi(u)$ is measurable in $0 < u < \infty$, then for any harmonic $P_g(x_j)$, if we write*

$$|x| = (x_1^2 + \ldots + x_k^2)^{1/2}, \quad |y| = (y_1^2 + \ldots + y_k^2)^{1/2}$$

and

$$l = \frac{k-2}{2}$$

we have

$$\frac{1}{(2\pi)^{k/2}} \int_{E_k} P_\varrho(x_j)\varphi(|x|)e^{i\Sigma x_j y_j}\, dv_x = \frac{i^\varrho P_\varrho(y_j)}{|y|^{k+2\varrho}} \int_0^\infty \varphi\left(\frac{t}{|y|}\right) J_{l+\varrho}(t)t^{l+\varrho+1}\, dt, \quad (5)$$

whenever the left side is absolutely convergent, say.

We introduce spherical coordinates

$$x_j = |x| \cdot \xi_j,\ \xi_1^2 + \ldots + \xi_k^2 = 1;\ y_j = |y| \cdot \eta_j,\ \eta_1^2 + \ldots + \eta_k^2 = 1,$$

which transforms the left side of (5) into

$$\frac{1}{(2\pi)^{l+1}} \int_0^\infty t^{\varrho+k-1}\varphi(t)\, dt \int_{S_{k-1}} e^{it|y|\cos\gamma} P_\varrho(\xi)\, d\omega_\xi, \quad (6)$$

where $\cos\gamma = \Sigma_j \xi_j \eta_j$ and $d\omega_\xi$ is the $(k-1)$-dimensional volume-element on the unit sphere S_{k-1} in E_k.

For $k = 2$, if we introduce ordinary polar coordinates $(|x|, \theta)$, $(|y|, \psi)$, then first of all we may assume $P_\varrho(\xi) = e^{i\varrho\theta}$, or $= e^{-i\varrho\theta}$. In the first case, (6) has the value

$$\int_0^\infty t^{\varrho+1}\varphi(t)\, dt \frac{1}{2\pi} \int_0^{2\pi} e^{i[t|y|\cos(\theta-\psi)+\varrho\theta]}\, d\theta,$$

and it is not hard to obtain from Bessel's integral for the function $J_\varrho(z)$ that the inner integral has indeed the value

$$(i)^\varrho e^{i\varrho\psi} J_\varrho(t|y|)$$

as claimed; and similarly in the second case.

For $k \geq 3$ we employ the expansion

$$e^{iz\cos\gamma} = 2^l\Gamma(l) \sum_{n=0}^\infty (n+l)i^n \frac{J_{n+l}(z)}{z^l} P_n^{\,l}(\cos\gamma),$$

where $P_n^{\,l}(\cos\gamma)$ is definable by

$$(1 - 2w\cos + w^2)^{-} = \sum w^n P_n^{\,l}(\cos\gamma),$$

and we note that for any given spherical harmonic $P_\varrho(\xi_j)$ the integral

$$\frac{\Gamma(l)(n+l)}{2\pi^{l+1}} \int_{S_{k-1}} P_n^{\,l}(\cos\gamma) P_\varrho(\xi)\, d\omega_\xi$$

has the value 0 if $n \neq g$, and the value $P_\varrho(\eta_j)$ if $n = g$;[4] which completes the proof of the theorem.

Another consequence of Theorem 1, by way of

$$\int_0^\infty e^{-pt} J_\nu(t)t^{\nu+1}\, dt = \frac{2^{\nu+1}p\Gamma\left(\nu+\frac{3}{2}\right)}{(1+p^2)^{\nu+(3/2)}\pi^{(1/2)}},$$

is

$$\int_{E_k} P_g(x_j)e^{-|y|}e^{i\Sigma x_j y_j}\,dv_x = P_g(y_j)\,\frac{i^g 2^k \pi^{(k-1)/2}\Gamma\left(\frac{k+1}{2}+g\right)}{(1+|y|^2)^{((k+1)/2)+g}};$$

and what is of much greater interest, by way of

$$\lim_{\epsilon\to 0}\int_0^\infty e^{-\epsilon t}J_\nu(t)t^{\mu-1}\,dt = \frac{2^{\mu-1}\Gamma\left(\frac{\mu+\nu}{2}\right)}{\Gamma\left(\frac{\mu+\nu}{2}+1\right)},$$

$Re(\mu+\nu) > 0$, the significant relation

$$\lim_{\epsilon\to 0}\frac{1}{(2\pi)^{k/2}}\int_{E_k} P_g(x_j)|x|^{s-g}e^{-\epsilon|x|+i\Sigma x_j y_j}\,dv_x = \frac{i^g P_g(y_j)}{|y|^{k+s+g}}\,\frac{2^{s+(k/2)}\Gamma\left(\frac{k+s+g}{2}\right)}{\Gamma\left(\frac{g-s}{2}\right)}, \quad (7)$$

for $|y| \neq 0$ ensues. The constant s may be complex. If we want the integral to be absolutely convergent we must put $Re(s) > -k$, but the relation also holds for $Re(s) > -k-g$, with the integral existing at the origin as a Cauchy principal value (spherical).

For $s = -k/2$ we obtain the remarkable formula

$$\lim_{\epsilon\to 0}\frac{1}{(2\pi)^{k/2}}\int_{E_k}\frac{P_g(\xi)}{|x|^{k/2}}e^{-\epsilon|x|+i\Sigma x_j y_j}\,dv_x = i^g\frac{P_g(\eta_j)}{|y|^{k/2}}$$

which assigns to the Fourier transform peculiar eigen-functions with eigen-values i^g. Also, rather more "pathological" than remarkable are the values $s-g = 0, 2, 4, \ldots$, the gamma function in the denominator being infinite, and the left side then having value 0 for $|y| \neq 0$.

We will terminate with a remark on the role of polynomials which are harmonic versus other ones. If in relation (4) we vary t but hold the other data fast then it has the general form

$$\sum_0^\infty a_m e^{-\lambda_m x} = x^{-\delta}\sum_0^\infty b_m e^{-(u_m/x)}, \quad (8)$$

$\delta > 0$; $\lambda_0 = \mu_0 = 0$, which we have recently studied,[5] and in this respect it does not differ from the parent relation (3) from which it can be obtained by differentiation; so that, in particular, it is equivalent with a functional equation so-called

$$\Gamma(s)\varphi(s) = \Gamma(\delta-s)\psi(\delta-s)$$

where

$$\varphi(s) = \sum_1^\infty \frac{a_m}{\lambda_m s}, \quad \psi(s) = \sum_1^\infty \frac{b_m}{\mu_m s},$$

just as the parent relation is. If however we differentiate (3) at random we will obtain a relation of the form

$$\sum_0^\infty a_m e^{-\lambda_m x} = \sum_{p=1}^q x^{-\delta_q} \sum_0^\infty b_m{}^q e^{-(\mu_m/x)},$$

but this still leads to a functional equation

$$\Gamma(s)\varphi(s) = \sum_{q=1}^p \Gamma(\delta_q - s)\psi_q(\delta - s)$$

where $\psi_q(s) = \sum_1^\infty b_m{}^q \mu_m{}^{-s}$. Thus, analytically, not everything is lost, and many other interesting features of relations of the form (8) will be retained. But what is apparently lost is the invariance with respect to a sizable subgroup of the modular group, and this seems to be the crux of the matter, arithmetically at least.

[1] His last paper subsuming previous ones is: "Analytische Arithmetik der positiven quadratischen Formen," *Kgl. Danske Videnskab. Selskab.*, **XVII**, 12 (1940).

[2] "Das Verhalten der mehrfachen Thetareihen bei Modulsubstitutionen," *Math. Ann.*, **116** 511–523, 780 (1939).

[3] Bochner, S., *Fouriersche Integrale*, p. 206.

[4] Bochner, S., and Taylor, A. E., "Some Theorems on Quasi-analyticity for Functions of Several Variables," *Am. J. Math.*, **61**, 303–329 (1939).

[5] "Some Properties of Modular Relations," *Ann. Math.*, **53**, 332–363 (1951).

*ON FINITE GROUPS WITH TWO INDEPENDENT GENERATORS. IV. CONJUGATE SUBSTITUTIONS**

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20. Conjugates θ of a Given Special Substitution φ.—Let φ be on the range n and have the period s; its derivative φ_1 is on the range $n_1 = s$, and will have a period s_1 that divides n. We suppose $\varphi_1(x)$ to be in *normal form*,[1] i.e., subject to the condition: $0 \leq \varphi_1(x) < s$.

Necessary and sufficient conditions for θ, on the range $m = su$, and φ, on the range n, to be conjugate special substitutions are:

$$\theta(0) \equiv 0 \pmod m, \qquad \varphi(0) \equiv 0 \pmod n \tag{20.1}$$

$$T_1\theta = \theta^{\varphi^t(1)}, \qquad T_1\varphi^y = \varphi^{\theta(y)} \tag{20.2}$$

$$\theta^n(1) \equiv 1 \pmod{m}, \qquad \varphi^m(1) \equiv 1 \pmod{n}. \tag{20.3}$$

(Cf. Note I, §3, and §7, Theorem VIII.)

The second conditions of (20.1), (20.3) are already secured. To secure the second condition of (20.2), we require

$$\theta(y) \equiv \varphi_1(y) \pmod{s},$$

or

$$\theta(x) \equiv \varphi_1(x) + s\psi(x) \pmod{m} \tag{20.4}$$

where $\psi(x)$ is an unknown function, whose residue mod u is alone of importance.

The first condition (20.1) demands that

$$\psi(0) \equiv 0 \pmod{u}. \tag{20.5}$$

Since θ must admit the translation T_s, the following condition is imposed on ψ:

$$\psi(x + s) \equiv \psi(x) + \psi(s) \pmod{u}. \tag{20.6}$$

Further,

$$g \equiv \theta(s)/s \equiv \psi(s) \pmod{u}, \tag{20.7}$$

so that

$$\psi(x + s) \equiv \psi(x) + g \pmod{u}, \tag{20.8}$$

and by induction on M,

$$\psi(x + Ms) \equiv \psi(x) + Mg. \tag{20.9}$$

g is subject to the condition

$$g^\epsilon \equiv 1 \pmod{u} \tag{20.10}$$

where

$$\epsilon = g.c.d.\ (\varphi^t(1) - 1, n), \qquad t = 1, 2, \ldots, s - 1. \tag{20.11}$$

Thus g is always prime to u.

Using (20.4), (20.9), we can compute the powers of θ, and find

$$\theta^k(x) \equiv \varphi_1{}^k(x) + g^{k-1}s \sum_{i=0}^{k-1} g^{-i}\psi\varphi_1{}^i(x) \pmod{m}. \tag{20.12}$$

Here g^{-i} is defined by the formula: $g^i \cdot g^{-i} \equiv 1 \pmod{u}$; its value can always be referred to a positive exponent by means of (20.10), i.e., by adding a multiple of ϵ to the exponent.

Formula (20.12) facilitates the application of the first condition in (20.2); with its use we obtain

$$\psi(x+t) - \psi(t) \equiv \sum_{i=0}^{k-1} g^{-i}\psi\varphi_1{}^i(x) + L(\varphi_1(x+t), \varphi_1(t)) \pmod{u} \tag{20.13}$$

where $k = \varphi^l(1)$, and $L(x, y) = 1$ if $x < y$, $L(x, y) = 0$ if $x \geqq y$.

Similarly, the first condition in (20.3) is expressed by

$$\frac{g^n - 1}{g^{s_1} - 1} \sum_{i=0}^{s_1-1} g^{-i}\psi\varphi_1{}^i(1) \equiv 0 \pmod{u}, \tag{20.14}$$

where, in accordance with our standing convention (cf. Note III, formula (16.12)), the fraction is to be interpreted as equal to n/s_1 in case $g = 1$.

In summary, necessary and sufficient conditions that θ defined by (20.4) be conjugate to the given φ are furnished by the system of simultaneous congruences (20.5, 8, 10, 13, 14) bearing on ψ as unknown function and g as unknown auxiliary quantity. It is sufficient to require (20.13) to hold for $x, t = 0, 1, 2, \ldots, s-1$, with formula (20.8) serving to prolong the function ψ outside this range, whereupon—as one may easily verify—the congruence (20.13) still remains in force for *all* x, t.

The period of θ is the smallest positive integer r, intermediate in divisibility between s_1 and n ($s_1 \mid r \mid n$), which is such that (20.14) holds when r is substituted for n. When $r = n$, and only then, φ is equal to the derivative θ_1 of θ, i.e., θ is an *antiderivative* of φ. To express this condition we must adjoin to our system of simultaneous congruences the incongruences

$$\frac{g^k - 1}{g^{s_1} - 1} \sum_{i=0}^{s_1-1} g^{-i}\psi\varphi_1{}^i(1) \not\equiv 0 \pmod{u}, \tag{20.15}$$

where $k = n/p$, p running through all prime factors of n/s_1. In case $g = 1$, the fraction preceding the summation sign is to be interpreted as equal to k/s_1.

The determination of the existence and the calculation of antiderivatives is of paramount importance in our theory, since if $\theta_1 = \varphi$, then θ is of the next higher type to φ. We thus have, at least theoretically, a procedure for obtaining substitutions of successively higher types. Of course, generally speaking, the system of congruences increases in complication and its solution in difficulty, as we proceed to higher types.

We may remark that *six* is the highest type of substitution we have so far been able to obtain explicitly. A specific example, θ, of this type, on the range 27, of period 18, will be given in a subsequent note of this series. The second derivative θ_2 of θ is the substitution (21.6), of type 4.

The interesting and difficult problem remains: *are there special substitutions θ of arbitrarily high type?* So far we have not been able to settle this question definitively.

21. Example (a).—Conjugates of

$$\varphi = (0)(2)(4)(135). \tag{21.1}$$

Here $n = 6$, $s = 3$, $m = 3u$, $\varphi_1 = (0)\ (12)$, $s_1 = 2$. φ is the simplest example of a type 3 substitution. After calculation, we find that our system of simultaneous congruences reduces to the following, all mod u:

$$g^2 \equiv 1 \tag{21.2}$$

$$3\psi(1) \equiv g - 2 \tag{21.3}$$

$$\psi(2) \equiv (g + 1)\psi(1) + g. \tag{21.4}$$

In case u is non-divisible by 3, the congruence (21.3) has a unique solution for $\psi(1)$, g being supposed previously chosen in accordance with (21.2). $\psi(2)$ can then be calculated by (21.4).

In case $3 \mid u$, we must have

$$g \equiv 2 \pmod 3 \tag{21.5}$$

in order that (21.3) may be solvable for $\psi(1)$:

$$\psi(1) \equiv (g - 2)/3 \pmod{u/3}.$$

Hence

$$\psi(1) \equiv \frac{g-2}{3} + \lambda\frac{u}{3} \pmod u, \qquad \lambda = 0, 1, -1;$$

and $\psi(2)$ may then be calculated by (21.4). g must be chosen previously in accord with (21.5) as well as (21.2).

Illustration: take $u = 3$, $g = 2$, $\lambda = 0$. Then $m = su = 9$; $\psi(1) \equiv 0$, $\psi(2) \equiv 2 \pmod 3$; $\theta(x) \equiv \varphi_1(x) + 3\psi(x) \pmod 9$.

$$\theta = (0)\ (127548)\ (36). \tag{21.6}$$

θ_1, the derivative of θ, $= \varphi$. θ is the simplest example of a special substitution of type 4.

The period r of any conjugate θ of (21.1) is intermediate in divisibility between $s_1 = 2$ and $n = 6$: $2 \mid r \mid 6$; hence $r = 2$ or 6. From this we see that any conjugate of φ is either an antiderivative of φ, therefore of type 4, or else is linear, hence of type 2. Thus φ has no conjugates of its own type (cf. Theorem X, Note I).

In order that the conjugate θ may be an antiderivative of φ, the incongruence

$$\psi(1) + g\psi(2) \not\equiv 0 \pmod u \tag{21.7}$$

is necessary and sufficient. Since from (21.2, 3, 4) we can infer

$$3(\psi(1) + g\psi(2)) \equiv 0 \pmod u \tag{21.7a}$$

[(21.3) times $g + 2$, plus (21.4) times $3g$], it follows that $3 \mid u$, or $9 \mid m$, is a necessary condition for any antiderivative of (21.1) [cf. (21.6)].

Example (*b*).—Conjugates of

$$\varphi = (0)(2)(4)(6)(1357). \tag{21.8}$$

Here $n = 8$, $s = 4$, $m = 4u$, $\varphi_1 = (0)(2)(13)$, $s_1 = 2$. We are able to reduce our system of simultaneous congruences to the following, all mod u:

$$\begin{aligned} &g^2 \equiv 1 \\ &2\psi(2) \equiv g - 1 \\ &(g - 1)\psi(3) + (g - 1) \equiv 0 \\ &(g + 1)\psi(2) + 4\psi(3) \equiv 3g - 1 \\ &\psi(1) \equiv g\psi(2) + \psi(3) - 1. \end{aligned} \tag{21.9}$$

θ will be an antiderivative of φ ($r = 8$) if and only if

$$(g + 1)\psi(2) \not\equiv 0 \pmod{u}. \tag{21.10}$$

Since from the first two congruences (21.9) we can infer

$$2(g + 1)\psi(2) \equiv 0 \pmod{u}, \tag{21.10a}$$

it follows that $2 \mid u$, or $8 \mid m$, is a necessary condition for an antiderivative. In point of fact, $m = 32$ is the minimum range of an antiderivative of (21.8) [cf. (21.12)]. For, if $g \equiv -1$, (21.10) is self-contradictory, while if $g \equiv 1$, (21.10) is in contradiction with the second of (21.9). Now, if $u = 2, 4, 6$, the only values of g that obey the necessary condition of primality to u are $g \equiv \pm 1 \pmod{u}$.

Illustration: take $u = 8$, $g = 3$; then $m = 32$, and $\psi(2) \equiv 1 \pmod 4$, $\psi(3) \equiv -1 \pmod 4$. Choose $\psi(2) = 1$, $\psi(3) = -1$; then $\psi(1) \equiv 1 \pmod 8$. By (20.4),

$$\theta(x) \equiv \varphi_1(x) + 4\psi(x) \pmod{32}.$$

For
$$x = 0, 1, 2, 3$$
$$4, 5, 6, 7$$
$$\text{etc.},$$

θ has the corresponding values:

$$\begin{array}{rcccc} \theta = & 0 & 7 & 6 & 29 \\ & 12 & 19 & 18 & 9 \\ & 24 & 31 & 30 & 21 \\ & 4 & 11 & 10 & 1 \\ & 16 & 23 & 22 & 13 \\ & 28 & 3 & 2 & 25 \\ & 8 & 15 & 14 & 5 \\ & 20 & 27 & 26 & 17 \end{array} \tag{21.11}$$

The numerals in each x-column form an arithmetic progression of difference $s = 4$, while each θ-column forms, modulo 32, an arithmetic progression of difference $gs = 12$. This state of affairs applies generally [see (20.4, 8)], so that the first row, corresponding to $0 \leqq x < s$, determines the entire table.

Expressed in cycles,

$$\begin{aligned}\theta = {} & (0)\,(16)\,(1\ 7\ 9\ 31\ 17\ 23\ 25\ 15) \\ & (3\ 29\ 27\ \ 5\ 19\ 13\ 11\ 21) \\ & (2\ 6\ 18\ 22)\,(10\ 30\ 26\ 14) \\ & (4\ 12)\,(8\ 24)\,(20\ 28). \end{aligned} \tag{21.12}$$

θ is obviously an antiderivative of φ, since the period $r = 8$ of θ is equal to the range of φ. φ being of type 3 [cf. Note II, §13, formula (131)], θ is an example of a type 4 substitution.

* For the previous notes of this series, see these PROCEEDINGS, **37**, 604–610, 677–691 749–760 (1951). A paper, covering some aspects of this work, with the title "On Finite Groups with Two Independent Generators," was read before the Academy, November 7, 1951.

[1] See Note I, §2.

PLANCHEREL FORMULA FOR COMPLEX SEMISIMPLE LIE GROUPS

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Let R and C be the fields of real and complex numbers respectively and let G be a connected (but not necessarily simply connected) complex semisimple Lie group and $\mathfrak{g}_0$ its Lie algebra over R. Let K be a maximal compact subgroup of G and let $\mathfrak{k}_0$ be the corresponding subalgebra of $\mathfrak{g}_0$. Define $\mathfrak{P}_0$, $\mathfrak{h}_{\mathfrak{k}_0}$, $\mathfrak{h}_{\mathfrak{P}_0}$ and $\mathfrak{h}_0$ as in a previous note.[1] Since G is a complex group there exists a 1-1 linear mapping Γ of $\mathfrak{k}_0$ on $\mathfrak{P}_0$ such that $[X, \Gamma(Y)] = \Gamma([X, Y])$ and $[\Gamma(X), \Gamma(Y)] = -[X, Y]$ $(X, Y \in \mathfrak{k})$. We extend Γ to a linear mapping of $\mathfrak{g}_0$ on itself by defining $\Gamma(\Gamma(X)) = -X (X \in \mathfrak{k}_0)$. Let $\sqrt{-1}$ be a fixed square root of -1 in C. For any $c \in C$ and $X \in \mathfrak{g}_0$ put $c * X = aX + b\Gamma(X)$ where $c = a + \sqrt{-1}\, b$ $(a, b \in R)$. Under this multiplication $\mathfrak{g}_0$ becomes a Lie algebra over C. We shall denote this complex algebra by $\mathfrak{g}^*$. Similarly the algebra $\mathfrak{h}_0$ regarded as a (complex) subalgebra of $\mathfrak{g}^*$ will be denoted by $\mathfrak{h}^*$. Then $\mathfrak{h}^*$ is a Cartan subalgebra of $\mathfrak{g}^*$. Let $X \to ad\, X (X \in \mathfrak{g}^*)$ be the adjoint representation of $\mathfrak{g}^*$ and let

$B(X, Y) = sp(ad\ X\ ad\ Y)$ $(X, Y \in \mathfrak{g}^*)$. Given any linear function λ on $\mathfrak{h}^*$ we denote by H_λ the unique element in $\mathfrak{h}^*$ such that $\lambda(H) = B(H, H_\lambda)$ for all $H \in \mathfrak{h}^*$. Let $H_1, \ldots, H_l$ be a base for $\mathfrak{h}_{\mathfrak{g}_0}$ over R. Then it is also a base for $\mathfrak{h}^*$ over C. We shall say that λ is real if $H_\lambda = \sum_{1 \leqq i \leqq l} c_i H_i (c_i \in R)$ and furthermore that $\lambda > 0$ if $\lambda \neq 0$ and $c_j > 0$ where j is the least index $(1 \leqq j \leqq l)$ such that $c_j \neq 0$. For every root α of $\mathfrak{g}^*$ (with respect to $\mathfrak{h}^*$) we choose an element $X_\alpha \neq 0$ in $\mathfrak{g}^*$ such that $[H, X_\alpha] = \alpha(H) * X_\alpha (H \in \mathfrak{h}^*)$. We can do this in such a way that $B(X_\alpha, X_{-\alpha}) = 1$ and $X_\alpha + X_{-\alpha}$, $\sqrt{-1} * (X_\alpha - X_{-\alpha})$ are both in $\mathfrak{v}_0$. Put $H_\alpha = \sum_{1 \leqq i \leqq l} \alpha^i H_i (\alpha^i \in R)$ and let $\mathfrak{N}^* = \sum_{\alpha \in P} C * X_\alpha$ where P is the set of all positive roots. Then $\mathfrak{N}^*$ is a nilpotent subalgebra of $\mathfrak{g}^*$ to which there corresponds an analytic subgroup N of G.

Let $C_c^\infty(G)$ be the class of complex-valued functions on G which are everywhere defined and indefinitely differentiable and which vanish outside a compact set. For any complex number c we denote by $\bar{c}$ its complex conjugate. Moreover if $z = x + \sqrt{-1}\, y$ $(x, y \in R)$ is a complex variable and f a complex-valued differentiable function of x and y, we write

$$\frac{\partial}{\partial z} f = \frac{1}{2}\left(\frac{\partial}{\partial x} - \sqrt{-1}\,\frac{\partial}{\partial y}\right) f, \quad \frac{\partial}{\partial \bar{z}} f = \frac{1}{2}\left(\frac{\partial}{\partial x} + \sqrt{-1}\,\frac{\partial}{\partial y}\right) f.$$

We shall now first prove a formula which has been obtained by Gelfand and Naimark[2] in the case when G is the $n \times n$ complex unimodular group. Let $X \to \exp X (X \in \mathfrak{g}_0)$ denote the exponential mapping of $\mathfrak{g}_0$ into G. Put $\rho = \frac{1}{2} \sum_{\alpha \in P} \alpha$ and let du and dn denote the elements of the invariant Haar measures on K and N respectively. We assume that $\int_K du = 1$.

THEOREM 1. *Put* $H_a = \sum_{1 \leqq i \leqq l} a_i * H_i (a_i \in C)$ *and*

$$D_\alpha = \sum_{1 \leqq i \leqq l} \alpha^i \frac{\partial}{\partial a_i}, \quad \bar{D}_\alpha = \sum_{1 \leqq i \leqq} \alpha \frac{\partial}{\partial \bar{a}_i} \quad (\alpha \in P).$$

Then with a suitable normalization of dn *we have*

$$f(1) = \lim_{H_a \to 0} \prod_{\alpha \in P} D_\alpha \bar{D}_\alpha \left\{ e^{\rho(H_a) + \overline{\rho(H_a)}} \int_{K \times N} f[u(\exp H_a) n u^{-1}]\, du\, dn \right\}$$

for any[3] $f \in C_c^\infty(G)$.

We give below a rapid sketch of the various steps leading to the proof of this theorem. Let θ denote the automorphism of $\mathfrak{g}_0$ over R given by $\theta(X + Y) = X - Y$ $(X \in \mathfrak{v}_0, Y \in \mathfrak{p}_0)$. Let $\mathfrak{N}_0$ denote the set $\mathfrak{N}^*$ regarded as a vector space over R and let $dX (X \in \mathfrak{N}_0)$ be the element of the usual Euclidean measure on $\mathfrak{N}_0$.

LEMMA 1. *There exists a real constant $c > 0$ such that*

$$\lim_{t \to 0} \frac{d}{dt} \left\{ e^{t\rho(H - \theta H)} \int_N f[(\exp tH)n]\, dn \right\} =$$

$$c \int_{\mathfrak{N}_0} \left\{ \frac{d}{dt} f[\exp(X + tH)] \right\}_{t=0} dX \qquad (t \in R)$$

for any $f \in C_c^\infty(G)$ and $H \in \mathfrak{b}_0$.

Since $\mathfrak{g}_0$ is a real Euclidean space it may be regarded as an analytic manifold. Let $C_c^\infty(\mathfrak{g}_0)$ be the class of all complex-valued functions on $\mathfrak{g}_0$ which are everywhere indefinitely differentiable and which vanish outside a compact set. Put

$$X = \sum_{1 \leq i \leq l} a_i * H_i + \sum_{\alpha \in P} z_\alpha * X_\alpha + \sum_{\alpha \in P} z_{-\alpha} * X_{-\alpha}$$

where a_i, z_α, $z_{-\alpha} (1 \leqq i \leqq l,\ \alpha \in P)$ are independent complex variables. For any complex variable $z = x + \sqrt{-1}\, y$ $(x, y \in R)$ let $d\mu(z)$ denote the element $dx\, dy$ of Euclidean measure on the corresponding complex plane. Let $x \to Ad(x)$ $(x \in G)$ be the adjoint representation of G. Consider a function $F \in C_c^\infty(\mathfrak{g}_0)$ such that $F(Ad(u)X) = F(X)$ $(u \in K)$. Put

$$g(Y) = \frac{1}{(2\pi)^n} \int_{\mathfrak{g}_0} \exp\left(\frac{1}{2}\sqrt{-1}\,[B(X, Y) + \overline{B(X, Y)}]\right) F(X)\, dX \qquad (Y \in \mathfrak{g}_0). \quad (1)$$

Here $n = \frac{1}{2} \dim_R \mathfrak{g}_0$ and $dX = \prod_{1 \leq i \leq l} d\mu(a_i) \prod_{\alpha \in P} d\mu(z_\alpha) d\mu(z_{-\alpha})$. Then if we assume, as we may, that $B(H_i, H_j) = \delta_{ij}$ $(1 \leqq i, j \leqq l)$ it follows that

$$F(0) = \frac{1}{(2\pi)^n} \int_{\mathfrak{g}_0} g(X)\, dX \qquad (2)$$

and $g(Ad(u)X) = g(X)$ $(u \in K, X \in \mathfrak{g}_0)$. Now it is known that $\bigcup_{u \in K} Ad(u)(\mathfrak{b}_0 + \mathfrak{N}_0) = \mathfrak{g}_0$ and from this we can deduce the following lemma.

LEMMA 2. *Let $g(X)$ be a measurable function on $\mathfrak{g}_0$ such that*

$$g[Ad(u)X] = g(X) \ (u \in K, X \in \mathfrak{g}_0) \text{ and } \int_{\mathfrak{g}_0} |g(X)|\, dX < \infty.$$

Then

$$\int_{\mathfrak{g}_0} g(X)\, dX = \int_{\substack{Z \in \mathfrak{N}_0 \\ H \in \mathfrak{t}_0}} \prod_{\alpha \in P} |\alpha(H)|^2 g(Z + H)\, dZ\, dH$$

where dZ and dH are the elements of the (suitably normalized) Euclidean measures on $\mathfrak{R}_0$ and $\mathfrak{h}_0$ respectively.

Applying this lemma to equation (2) we get

$$F(0) = \lim_{H_a \to 0} \left\{ c \int_{Z \in \mathfrak{R}_0} \prod_{\alpha \in P} D_\alpha \bar{D}_\alpha F(Z + H_a)\, dZ \right\}$$

where c is a positive real constant depending only on the normalization of dZ. The assertion of the theorem now follows without much difficulty if we take into account lemma 1.

Now we assume that the base $H_1, \ldots, H_l$ is so chosen that $\exp H_a = 1$ if and only if $\sqrt{-1}\,\frac{a_i}{2\pi}\,1 \leqq i \leqq l$ are all rational integers. Let A, A_+ and A_- be the analytic subgroups of G corresponding to $\mathfrak{h}_0$, $\mathfrak{h}_{\mathfrak{P}_0}$ and $\mathfrak{h}_{\mathfrak{k}_0}$ respectively. Then A_- is compact while A_+ is simply connected. For any $h \in A$ we denote by h_+ and h_- the unique elements in A_+ and A_- respectively such that $h = h_+h_-$. Also let $\log h_+$ denote the unique element $H \in \mathfrak{h}_{\mathfrak{p}_0}$ such that $h_+ = \exp H$. Let $\tilde{\mathfrak{F}}_+$ be the set of all linear functions ν on $\mathfrak{h}^*$ such that $\nu(H)$ is real for all $H \in \mathfrak{h}_{\mathfrak{P}_0}$. Moreover let $\tilde{\mathfrak{F}}_-$ denote the set of all linear functions Λ on $\mathfrak{h}^*$ such that $\Lambda(H_i)1 \leqq i \leqq l$ are all integers. Given any $\nu \in \tilde{\mathfrak{F}}_+$ and $\Lambda \in \tilde{\mathfrak{F}}_-$ put

$$\xi_{\nu,\Lambda}(h) = e^{\sqrt{-1}\,\nu(\log h_+)} e^{\Lambda(\log h_-)} \qquad (h \in A)$$

$\log h_-$ being any element in $\mathfrak{h}_{\mathfrak{k}_0}$ such that $\exp(\log h_-) = h_-$. It is known that the mapping $(u, h, n) \to uhn (u \in K, h \in A_+, n \in N)$ is a topological mapping of $K \times A_+ \times N$ on G. We may normalize the Haar measure on G in such a way that

$$dx = e^{4\rho(\log h)}\, du\, dh\, dn \qquad (x = uhn, u \in K, h \in A_+, n \in N)$$

dh being the Haar measure on A_+. Moreover we assume that the normalization of the various Haar measures is such that Theorem 1 holds and

$$\int_K du = 1,\ \int_{A_-} dh_- = 1,\ dh = dh_+dh_- \qquad (h \in A).$$

For any $x \in G$ and $u \in K$ define $u_x \in K$ and $H(x, u) \in \mathfrak{h}_{\mathfrak{P}_0}$ by the relation

$$xu = u_x[\exp H(x, u)]n \qquad (n \in N).$$

Given any $\Lambda \in \tilde{\mathfrak{F}}_-$ let $\mathfrak{H}_\Lambda'$ denote the set of all continuous functions ψ on K such that

$$\psi(u \exp H) = e^{-\Lambda(H)}\psi(u)\ (u \in K, H \in \mathfrak{h}_{\mathfrak{k}_0}).$$

Let $L_2(K)$ be the Hilbert space consisting of all measurable and square-integrable functions on K, taken with the usual norm. Then the closure $\mathfrak{H}_\Lambda$ of $\mathfrak{H}_\Lambda'$ in $L_2(K)$ is a Hilbert space. For any $\nu \in \tilde{\mathfrak{F}}_+$ we define a unitary

representation $\pi_{\nu,\Lambda}$ of G on $\mathfrak{H}_\Lambda$ as follows. If $\psi \in \mathfrak{H}_\Lambda$ its transform $\pi_{\nu,\Lambda}(x)\psi = \varphi$ is given by

$$\varphi(u) = e^{-\sqrt{-1}\nu(H(x^{-1},u))}e^{-2\rho(H(x^{-1},u))}\psi(u_{x^{-1}}) \qquad (u \in K).$$

It is easily proved that if $f \in C_c^\infty(G)$, the operator

$$\int_G f(x)\pi_{\nu,\Lambda}(x)\,dx$$

has a trace $T_{\nu,\Lambda}(f)$ which is given by

$$T_{\nu,\Lambda}(f) = \int f(uhnu^{-1})\xi_{\nu,\Lambda}(h)e^{2\rho(\log h,)}\,du\,dh\,dn$$

where the integral extends over all $u \in K$, $h \in A$, $n \in N$. Now $\mathfrak{F}+$ is clearly a vector space over R of finite dimension. Let $d\nu$ denote the element of Euclidean measure in $\mathfrak{F}+$. Then the following result is easily obtained from Theorem 1.

THEOREM 2. *Put*

$$m(\nu,\Lambda) = \prod_{\alpha \in P} \left|\sqrt{-1}\,\nu(H_\alpha) + \Lambda(H_\alpha)\right|^2 \qquad (\nu \in \mathfrak{F}+,\ \Lambda \in \mathfrak{F}-).$$

Then if $d\nu$ is suitably normalized we have the formula

$$f(1) = \sum_{\Lambda \in \mathfrak{F}-} \int_{\mathfrak{F}+} m(\nu,\Lambda)T_{\nu,\Lambda}(f)\,d\nu \qquad [f \in C_c^\infty(G)]$$

the series being absolutely convergent.

Now suppose $f \in C_c^\infty(G)$ and

$$F(x) = \int_G \overline{f(y)}f(yx)\,dy.$$

Then $F \in C_c^\infty(G)$ and the operator $\int_G F(x)\pi_{\nu,\Lambda}(x)\,dx$ is self-adjoint and positive semidefinite. Hence $T_{\nu,\Lambda}(F)$ is real and non-negative. In fact

$$T_{\nu,\Lambda}(F) = \int_{v,\,u \in K} \left|f_{\nu,\Lambda}(v,u)\right|^2 dv\,du$$

where

$$f_{\nu,\Lambda}(v,u) = \int_{AN} f(vhnu^{-1})\xi_{\nu,\Lambda}(h)e^{2\rho(\log h,)}\,dh\,dn.$$

Therefore

$$\int_G \left|f(x)\right|^2 dx = F(1) = \sum_{\Lambda \in \mathfrak{F}-} \int_{\mathfrak{F}+} m(\nu,\Lambda)\,d\nu \int_{K \times K} \left|f_{\nu,\Lambda}(v,u)\right|^2 dv\,du$$

from Theorem 2. Since $m(\nu,\Lambda)$ is real and non-negative the following analogue of the Plancherel theorem is now easily obtained.

THEOREM 3. *Let f be a measurable function on G such that*

$$\int_G \left|f(x)\right|^2 dx < \infty \text{ and } \int_G \left|f(x)\right| dx < \infty.$$

Then

$$\int_G |f(x)|^2\,dx = \sum_{\Lambda \in \tilde{\Omega}} \int_{\tilde{\Omega}_\Lambda} m(\nu, \Lambda)\,d\nu \int_{K \times K} dv\,du \left| \int_{AN} f(vhnu^{-1})\,\xi_{\nu,\Lambda}(h) e^{2\rho(\log h_+)}\,dhdn \right|^2$$

[1] Harish-Chandra, PROC. NATL. ACAD. SCI., **37**, 362–365 (1951).
[2] Gelfand and Naimark, *Trudi Mat. Inst. Steklova*, **36**, 198 (1950).
[3] We denote the unit element of G by 1.

ALTERNATIVE DIVISION RINGS OF CHARACTERISTIC 2

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Alternative, not associative, division rings of characteristic other than 2 have recently been investigated.[1-3] The present paper completes this investigation by showing that *every alternative, not associative division ring R of characteristic 2 is a Cayley-Dickson division algebra of order 8 over its center.*

The idea of proving this result by means of Lemma 4 stems from the work of L. A. Skornyakov.[3] The methods employed are those of R. H. Bruck and the author.[1, 2] This combination disposes of the characteristic 2 case in relatively short order. Even though the present calculations are rather special, they do indicate how L. A. Skornyakov's paper[3] may be considerably shortened.

The value of the *associator* $(x, y, z) = (xy)z - x(yz)$ remains unchanged by any permutation of its components. This property is also shared by the *commutator* $(x, y) = xy - yx$ and $f(w, x, y, z)$, defined by

$$(wx, y, z) = f(w, x, y, z) + x(w, y, z) + (x, y, z)w, \qquad (1)$$

where w, x, y, z are elements in R.[1, 2] Moreover the following two identities hold:

$$f(w, x, y, z) = ((w, x), y, z) + ((y, z), w, x), \qquad (2)$$

$$(xy, z) = x(y, z) + (x, z)y + (x, y, z). \qquad (3)$$

Our calculations will be based almost entirely on (1), (2) and (3).

If $(x, y, R) = 0$ whenever the elements x, y of R commute, then it follows[2] that R is a Cayley-Dickson algebra over its center. However in a Cayley-Dickson algebra of characteristic 2 we can always find elements x, y, z

such that $(x, y) = 0$ and $(x, y, z) \neq 0$. Consequently there also exist such elements, x, y, z in R. Let $v = (x, y, z)$. Then $(v, x) = (v, y) = (v, x, y) = 0$. In view of (3), $(vz, x) = v(z, x) + (v, z, x)$. But $v(z, x) = (v, z, x)$. Hence $(vz, x) = 0$ and similarly $(vz, y) = 0$. Because of (1), $(zv, x, y) = v^2 + f(v, z, x, y)$. But use of (2) yields $f(v, z, x, y) = (vz + zv, x, y)$. Therefore $(vz, x, y) = v^2 \neq 0$. Let $x = a$, $y = b$, $vz = c$. Then $(a, b) = (a, c) = (b, c) = 0$, while $u = (a, b, c) \neq 0$. We denote by $S = S[a, b, c]$ the subring of R generated by a, b, c, and by K the set of all k in R such that $(k, S) = 0 = (k, S, S)$.

LEMMA 1. *If $p \in R$ and $(p, a, b) = (p, a, c) = (p, b, c) = 0$ then $p \in K$.*

The use of (2) makes $f(p, a, b, c) = ((p, a), b, c)$. In view of (1), $f(p, a, b, c) = (pa, b, c) + (a, b, c)p$. By comparison, $up = (a, b, c)p = (ap, b, c)$. However $b(ap, b, c) = (ap, b, cb) = (ap, b, bc) = (ap, b, c)b$, so that $(up, b) = 0$. By the definition of u, $(u, p, b) = (ba \cdot c, p, b) + (b \cdot ac, p, b)$. With the help of (1) and hypothesis, $(ba \cdot c, p, b) = f(ba, c, p, b)$. On the other hand use of (2) gives $f(ba, c, p, b) = ((p, c), ba, b) = ((p, c), a, b)b = f(p, c, a, b)b$. Consequently $(u, p, b) = f(p, a, b, c)b + (ac, p, b)b = f(p, a, b, c)b + f(p, a, b, c)b = 0$. Use of (3) gives $0 = (up, b) = u(p, b) + (u, b)p + (u, p, b) = u(p, b)$. Since $u \neq 0$, $(p, b) = 0$, and in similar fashion it may be shown that $(p, a) = (p, c) = 0$. This is sufficient to prove the lemma.[2]

As a consequence of Lemma 1 it is readily verified that $a^2, b^2, c^2, u \in K$. Furthermore identities like $(ab, c) = u$, $(ab, ac) = 0$ and others obtained by suitable permutations of a, b, c permit us to set up a multiplication table for the elements $1, a, b, c, ab, ac, bc, a \cdot bc$, which shows that these eight elements behave like basis elements of the Cayley-Dickson algebra of characteristic 2.

LEMMA 2. *K is a field.*

To prove this assertion it is sufficient to show that K is closed under multiplication and inverses, and that K is associative and commutative. Let k, k' be in K. Then $(kk', a, b) = f(k, k', a, b)$, using (1). Because of (2), $f(k, k', a, b) = ((k, a), k', b) + ((k', b), k, a) = 0$. Therefore use of Lemma 1 shows that kk' is in K. If $k \neq 0$, then $0 = k^{-1}(k, a, b) = f(k, k^{-1}, a, b) + (k^{-1}, a, b)k$. However $f(k, k^{-1}, a, b) = ((k, k^{-1}), a, b) + ((a, b), k, k^{-1}) = 0$, so that $(k^{-1}, a, b)k = 0$ and hence $(k^{-1}, a, b) = 0$. Again by Lemma 1, k^{-1} is shown to be in K. Let s be an arbitrary element of S. Then $0 = (kk', s) = (k, k', s)$ by (3). Also $(ak, b, c) = f(a, k, b, c) + k(a, b, c)$ using (1), while $f(a, k, b, c) = ((a, k), b, c) + ((b, c), a, k) = 0$ using (2), so that $(ak, b, c) = k(a, b, c)$. On the other hand $f(ak', k, b, c) = ((ak', b), k, c)$ in view of (2), while $(ak', b) = a(k', b) + (a, b)k' + (a, k', b) = 0$ by (3), so that $f(ak', k, b, c) = 0$. Therefore $kk'(a, b, c) = k(ak', b, c) = (ak'k, b, c) = k'k(a, b, c)$. Consequently $(k, k')(a, b, c) = 0$. Since $(a, b, c) \neq 0$ we obtain $(k, k') = 0$. This shows K to be commutative

and now with the use of (3) K may be shown to be associative and the lemma is proved.

The essence of the proof of our result lies in showing that any element d in R may be written as $d = \beta_0 + \beta_1 a + \beta_2 b + \beta_3 c + \beta_4 ab + \beta_5 ac + \beta_6 bc + \beta_7 a \cdot bc$, where $\beta_0, \ldots, \beta_7 \in K$. This is done in the following two lemmas.

LEMMA 3. *If* $(d, a) = 0 = (d, b)$, *then* $d = \alpha_0 + \alpha_1 a + \alpha_2 b + \alpha_3 c + \alpha_4 ab$, *for some* $\alpha_0, \ldots, \alpha_4$ *in* K.

We begin by showing that (d, c), (d, ab), (d, ac), (d, bc) are elements of K. Suppose that $(w, z) = (x, y) = (y, z) = 0$. In view of (2), $((w, x), y, z) = f(w, x, y, z) = ((w, z), x, y) + ((x, y), w, z) = 0$. Therefore $((d, c), a, b) = ((d, c), a, c) = ((d, c), b, c) = 0$ and (d, c) is an element of K. By the same reasoning we can show that (d, ac), (d, bc) are elements of K and $((d, ab), a, b) = 0$. Suppose that $(c, s) = (s, t) = 0$, where s, t are in S. Then in view of (2), $((d, t), s, c) = f(d, t, s, c) = ((c, d), s, t) + ((s, t), c, d) = 0$. Consequently $((d, ab), a, c) = ((d, ab), b, c) = 0$ and we see that (d, ab) is an element of K. Now form $d' = d - [(d, c)u^{-1}]ab - [(d, ab)u^{-1}]c - [(d, ac)u^{-1}]b - [(d, bc)u^{-1}]a$. Using the multiplication table for the elements $a, b, c, ab, ac, bc, a \cdot bc$ together with (3) and Lemma 2, we obtain $(d', a) = (d', b) = (d', c) = (d', ab) = (d', ac) = (d', bc) = 0$. Thus, in view of (3), $(d', a, b) = (d', a, c) = (d', b, c) = 0$, so that d' is an element of K by Lemma 1. If we take $\alpha_0 = d'$, $\alpha_1 = (d, bc)u^{-1}$, $\alpha_2 = (d, ac)u^{-1}$, $\alpha_3 = (d, ab)u^{-1}$, $\alpha_4 = (d, c)u^{-1}$, then the lemma is proved.

LEMMA 4. *Any element* d *of* R *may be written as* $d = \beta_0 + \beta_1 a + \beta_2 b + \beta_3 c + \beta_4 ab + \beta_5 ac + \beta_6 bc + \beta_7 a \cdot bc$, *for some* $\beta_0, \ldots, \beta_7$ *in* K.

Use of (1) gives $du = d(a, b, c) = (a, b, cd) + f(a, b, c, d) + (a, b, d)c$. On the other hand use of (2) gives $f(a, b, c, d) = (a, b, cd + dc)$. Hence $d(a, b, c) = (a, b, dc) + (a, b, d)c$. We note that both (a, b, dc) and (a, b, d) satisfy the hypothesis of Lemma 3. This is sufficient to establish the present Lemma.

We are now ready to prove the main result. Clearly the center of R can be shown to coincide with K, in view of Lemma 4. In fact R is a finite dimensional algebra over K. As simple alternative, not associative algebras are completely classified,[4] we obtain the desired result. However it is not difficult to show by direct computation that the elements $1, a, b, c, ab, ac, bc, a \cdot bc$, are linearly independent over K, and thus are a set of basis elements for the Cayley-Dickson division algebra R over K.

[1] Bruck, R. H., and Kleinfeld, E., PROC. NATL. ACAD. SCI., **37**, 88–90 (1951).

[2] Bruck, R. H., and Kleinfeld, E. (in press, *Proc. Am. Math. Soc.*).

[3] Skornyakov, L. A., *Ukrain. Mat. Zurnal*, **2**, 70–85 (1950).

[4] Schafer, R. D., *Bull. Am. Math. Soc.*, **49**, 549–555 (1943).

NOTE ON APPROXIMATION BY BOUNDED ANALYTIC FUNCTIONS

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The primary object of this note is to establish a result on the topic indicated in the title:

THEOREM 1. *Let C be an analytic Jordan curve, which together with its interior lies in a region D. Let the sequence of functions $f_n(z)$ be analytic in D, satisfy the inequality ($R > 1$)*

$$|f_n(z)| \leqq A_1 R^n, \; z \text{ in } D, \tag{1}$$

and likewise for some function $f(z)$ satisfy the inequality

$$|f(z) - f_n(z)| \leqq A_2/n^{p+\alpha}, \; z \text{ on } C, \tag{2}$$

where p is a non-negative integer and we have $0 < \alpha \leqq 1$; here and below, all numbers A_j represent positive constants, independent of n and z. Then the pth derivative $f^{(p)}(z)$ satisfies on C a Lipschitz condition of order α if we have $0 < \alpha < 1$, and is of class Λ^* with respect to arc length on C if we have $\alpha = 1$.

We assume that D is a Jordan region bounded by a curve C_ρ, namely the image of the circle γ_ρ: $|w| = \rho(>1)$ when the exterior of C is mapped conformally by $z = \psi(w)$ onto the exterior of γ: $|w| = 1$, so that the points at infinity correspond to each other; this assumption obviously does not diminish the generality of the proof. Let $P_0(z)$, $P_1(z)$, $P_2(z)$, ..., be the Faber polynomials for C; any function $F(z)$ analytic and bounded interior to C_ρ can be expanded interior to C_ρ:

$$F(z) \equiv \sum_{k=0}^{\infty} a_k P_k(z), \quad a_k = \frac{1}{2\pi i} \int_{\gamma_\rho} \frac{F[\psi(w)]\, dw}{w^{k+1}}, \tag{3}$$

where in the integral the Fatou boundary values are used on γ_ρ. The polynomials $P_k(z)$ are uniformly bounded on C.

Thus if we write

$$f_n(z) \equiv a_{n0}P_0(z) + a_{n1}P_1(z) + a_{n2}P_2(z) + \ldots, \tag{4}$$

we have by (1) and (3)

$$|a_{nk}| \leqq A_1 R^n/\rho^k,$$

and if $S_{n,N}(z)$ denotes the sum of the first $N+1$ terms of the second member of (4), we have by the boundedness of the $P_n(z)$ on C,

$$|f_n(z) - S_{n,N}(z)| \leqq \sum_{k=N+1}^{\infty} A_3 R^n/\rho^k = A_4 R^n/\rho^N, \; z \text{ on } C. \tag{5}$$

We now choose the positive integer λ so that $\rho^\lambda > R$, whence

$$|f_n(z) - S_{n,\lambda n}(z)| \leqq A_4(R/\rho^\lambda)^n, \ z \text{ on } C, \tag{6}$$

with $R/\rho^\lambda < 1$. From (2) and (6) we may write

$$|f(z) - S_{n,\lambda n}(z)| \leqq A_5/n^{p+\alpha}, \ z \text{ on } C. \tag{7}$$

The polynomials $S_{n,\lambda n}$ of respective degrees λn are not defined for every degree, but we may set

$$p_k(z) \equiv S_{n,\lambda n}(z), \ \lambda n \leqq k < \lambda(n+1).$$

Then the polynomials $p_k(z)$ are defined for all positive integral values of k, we have from (7)

$$|f(z) - p_k(z)| \leqq A_6/k^{p+\alpha}, \ z \text{ on } C, \tag{8}$$

and the conclusion follows[1, 2] from (8).

In determining the polynomials $S_{n,\lambda n}(z)$ of degrees λn in (6) we have used for convenience the Faber polynomial expansion of the given functions $f_n(z)$. Various other polynomial expansions may also be used, not necessarily of the form $\Sigma c_k P_k(z)$ in terms of given polynomials $P_k(z)$. It is sufficient if there can be determined polynomials $S_{n,\lambda n}(z)$ of respective degrees λn approximating on C to an arbitrary function $f_n(z)$ analytic and in modulus not greater than M_n interior to C_ρ, with measure of approximation indicated by the inequality

$$|f_n(z) - S_{n,\lambda n}(z)| \leqq A_7 M_n/\rho^{\lambda n}, \ z \text{ on } C.$$

Numerous kinds of polynomials, defined for instance by interpolation, satisfy this condition;[3] it is essential that C possess suitable continuity properties, but C need not be analytic; Theorem 1 admits a corresponding extension.

To Theorem 1 we add an easily established converse:

THEOREM 2. *Let C be an analytic Jordan curve, which together with its interior lies in a bounded region D. Let $f(z)$ be analytic interior to C, continuous in the corresponding closed region, and let $f^{(p)}(z)$ satisfy on C a Lipschitz condition of order $\alpha (0 < \alpha < 1)$ or be of class Λ^* with respect to arc-length on C $(\alpha = 1)$. Then there exist polynomials $f_n(z)$ of respective degrees n so that (1) and (2) are satisfied.*

The existence of polynomials $f_n(z)$ satisfying (2) is known.[1, 2] For these polynomials we have by (2) and by the boundedness of $f(z)$ on C

$$|f_n(z)| \leqq A_8, \ z \text{ on } C. \tag{9}$$

We choose $\rho (>1)$ so large that, in the notation already introduced, D lies interior to C_ρ. Then (9) implies by the generalized Bernstein lemma[4]

$$|f_n(z)| \leqq A_8\rho^n, \ z \text{ interior to } C_\rho,$$

so (1) follows with $R = \rho$.

The discussion just given also includes the

COROLLARY. *If the polynomials $f_n(z)$ of respective degrees n are given so that (2) is valid, and if D is bounded, then (1) is also valid.*

If C, D, $f(z)$ satisfy the conditions of Theorem 1, and if a positive number M is given, it follows from Montel's theory of normal families *that among the functions analytic and in modulus not greater than M in D, there exists at least one function $F_M(z)$ of best approximation to $f(z)$ on C*, namely, such that

$$\mu_M = \max. [|f(z) - F_M(z)|, z \text{ on } C] \tag{10}$$

is least. Theorems 1 and 2 are of significance in the study of such functions:

THEOREM 3. *Let D be a bounded region containing in its interior the closed interior of the analytic Jordan curve C, and let the function $f(z)$ be analytic interior to C, continuous in the corresponding closed region. For each positive M, let $F_M(z)$ be the (or a) function analytic and of modulus not greater than M in D, of best approximation to $f(z)$ on C in the sense that μ_M given by (10) is least. Then a necessary and sufficient condition that $f^{(p)}(z)$ satisfy a Lipschitz condition of order $\alpha (0 < \alpha < 1)$ on C or be of class Λ^* $(\alpha = 1)$ for arc-length on C is that*

$$\log M \cdot {\mu_M}^{1/(p+\alpha)} \tag{11}$$

be bounded as $M \to \infty$.

If (11) is bounded, we choose the sequence $M = e^n$, whence for the functions $F_M(z)$ and a suitably chosen A_9

$${\mu_M}^{1/(p+\alpha)} \leqq A_9/n, \ \mu_M \leqq {A_9}^{p+\alpha}/n^{p+\alpha},$$

and the conclusion follows from Theorem 1.

Conversely, let $f(z)$ be given with the requisite properties on C. We compare the measure of approximation μ_M with the corresponding measure of approximation for the polynomial $f_n(z)$ of Theorem 2, for specific values of M not less than the modulus of $f_n(z)$ in D. We obviously have $\mu_M \leqq A_2/n^{p+\alpha}$, provided n is defined as a function of M by the inequality $A_1 R^n \leqq M < A_1 R^{n+1}$, whence

$${\mu_M}^{1/(p+\alpha)} \leqq {A_2}^{1/(p+\alpha)}/n, \log M < \log A_1 + (n+1) \log R,$$

from which it follows that (11) is bounded. Theorem 3 is established.

An alternate form of the condition on (11) is to require that

$$\log \mu_M + (p + \alpha) \log \log M$$

be bounded as M becomes infinite. If (for $0 < \alpha < 1$) the function $f^{(p)}(z)$

satisfies a Lipschitz condition of order α but of no greater order, or (for $\alpha = 1$) if $f^{(p)}(z)$ is of class Λ^* but $f^{(p+1)}(z)$ does not satisfy a Lipschitz condition of any order, *then* $p + \alpha$ *is the greatest number* γ *for which*

$$\log \mu_M + \gamma \log \log M \tag{12}$$

is bounded as $M \to \infty$, where μ_M is the measure of approximation for the function $F_M(z)$ of best approximation. Indeed, $p + \alpha$ is the greatest number γ for which (12) is bounded as M becomes infinite, not passing through all values but passing through an arbitrary sequence of values of the form $M = A_0 R_1^n$, $n = 1, 2, 3, \ldots$.

Alternative (and simpler) proofs of Theorem 1, and hence of Theorem 3 can be given by mapping the *interior* of C onto the interior of $\gamma: |w| = 1$, and making use of the special properties of the Taylor development in the closed interior of γ. Of course for the unit circle the Faber development is identical with the Taylor development, so this alternate proof of Theorem 1 is not greatly different in form from the proof already given. This alternate proof has no immediate analog, however, if we replace the Jordan curve C by a Jordan arc.

Theorems 1, 2 and 3 extend to the case where C is an analytic Jordan arc.

THEOREM 4. *Let* C *be an analytic Jordan arc, let* D *be a region containing* C, *and let the functions* $f_n(z)$ *be analytic in* D *and satisfy both* (*1*) *and* (*2*). *Then* $f^{(p)}(z)$ *satisfies a Lipschitz condition of order* α *if* $0 < \alpha < 1$ *and is of class* Λ^* *for arc-length on* C *if* $\alpha = 1$, *on any closed subarc of* C *containing no endpoint of* C.

Map C conformally by $z = \varphi(w)$ onto the line segment S: $-1 \leqq w \leqq 1$; conditions (1) and (2) in suitably modified form persist. We approximate in the w-plane on S to the functions $f_n[\varphi(w)]$ by polynomials in w which interpolate to $f_n[\varphi(w)]$ in the zeros of the Tchebycheff polynomials for S. The second (outlined) proof of (6) making use of polynomials defined by interpolation is then valid.[3] Introduce now the classical transformation $w = \cos\theta$ which maps S onto the axis of reals $-\infty < \theta < \infty$ and transforms a polynomial in w of degree n into a trigonometric polynomial in θ of order n. From a theorem due to S. Bernstein and de la Vallée Poussin in the case $0 < \alpha < 1$ and from a theorem due to Zygmund in the case $\alpha = 1$, it now follows that $f[\varphi(\cos\theta)]$ has the requisite properties as a function of θ. The class Λ^* is invariant under suitable transformation,[2] so Theorem 4 follows.

If C is of length $2c$, and arc-length s is suitably measured on C algebraically from the mid-point, it readily follows by a slight extension of the reasoning just given, that the function $f(z) \equiv F(s/c) \equiv F(\cos\omega)$ has on C a pth derivative with respect to ω which satisfies a Lipschitz condition of order $\alpha (0 < \alpha < 1)$ or is of class $\Lambda^* (\alpha = 1)$ with respect to ω. The reciprocal of this reasoning is also valid, including the proof of (1) and (2).

THEOREM 5. *Let C be an analytic Jordan arc which lies in a bounded region D, and let the function $f(z)$ be given on C. For each positive M, let $F_M(z)$ be the (or a) function analytic and of modulus not greater than M in D, whose measure of approximation to $f(z)$ on C as given by (10) is least. Let C be of length $2c$ and let arc-length s on C be measured algebraically from the mid-point; we set $f(z) \equiv F(s/c) \equiv F(\cos \omega)$ on C. Then a necessary and sufficient condition that $d^p F(\cos \omega)/d\omega^p$ satisfy a Lipschitz condition of order $\alpha (0 < \alpha < 1)$ or be of class $\Lambda^*(\alpha = 1)$ with respect to ω is that (11) be bounded as M becomes infinite.*

A further result is similar to Theorems 4 and 5:

THEOREM 6. *Let $f(x)$ be a real or complex-valued function of the real variable x, periodic with period 2π. Let $d(>0)$ be arbitrary but fixed. For each M (>0), among the functions analytic and in modulus not greater than M in the region D: $|y| < d$, periodic with period 2π, let $F_M(z)$ denote the (or a) function of best approximation to $f(x)$ on C: $-\infty < x < \infty$ in the sense that*

$$\mu_M = lub[\,|f(x) - F_M(x)|\,,\ -\infty < x < \infty\,]$$

is least. Then a necessary and sufficient condition that $f^{(p)}(x)$ satisfy a Lipschitz condition of order α $(0 < \alpha < 1)$ or be of class Λ^* $(\alpha = 1)$ on C is that (11) be bounded as $M \to \infty$.

The sufficiency of this condition follows from Theorem 4 or Theorem 5 To prove the necessity of the condition, we employ as comparison sequence as in the proof of Theorem 3, the known sequence of trigonometric polynomials $f_n(z)$ of respective orders n which satisfy (2). For this sequence inequality (1) is readily established by setting $w = e^{iz}$, which maps C onto the circumference $|w| = 1$: a trigonometric polynomial in z of order n is a polynomial in w and $1/w$ of degree n, and it is convenient to apply a lemma[5] concerning the latter type of polynomial. Theorem 6 remains valid if D is no longer the region $|y| < d$ but a subregion of the latter containing C, and also remains valid if the $F_M(z)$ are not required to be periodic.

If we modify the hypothesis of Theorem 1 by merely requiring that C be a continuum (not a single point) interior to D, and by replacing the second member of (2) by A_2/R_2^n, $R_2 > 1$, it follows that the sequence $f_n(z)$ converges uniformly and its limit $f(z)$ is analytic in some subregion of D containing C [compare Walsh, *Trans. Am. Math. Soc.*, **47,** 293–304 (1940)]. A similar remark applies to Theorems 4 and 6.

The results of the present note concern Problem α, namely the study of the connection between measure of approximation to $f(z)$ on C and continuity properties of $f(z)$ on C. A further study involves Problem β, the connection between measure of approximation to $f(z)$ on C and continuity properties of $f(z)$ on a related point set such as C_ρ; results on the latter problem will be forthcoming.

[1] Sewell, W. E., "Degree of Approximation by Polynomials in the Complex Domain," *Ann. Math. Studies*, **9** (Princeton, 1942).

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ON A THEOREM OF HARDY, LITTLEWOOD, POLYA, AND BLACKWELL

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Let $U = \{u\}$ be a real vector space. Let $a(b)$ be a measure on U with a finite spectrum, $S_a = \{u_i \mid 1 \leq i \leq n_a\}$ ($S_b = \{u^j \mid 1 \leq j \leq n_b\}$). We say that

$$a \underset{1}{>} b$$

if and only if for each real convex $\varphi \mid U$

$$\sum_i a(u_i)\varphi(u_i) \geqq \sum_j b(u^j)\varphi(u^j).$$

We say that

$$a \underset{2}{>} b$$

if and only if

$$b(u^j)u^j = \sum_i p_{ij}a(u_i)u_i,$$

where

$$p_{ij} \geqq 0,$$
$$\sum_i p_{ij}a(u_i) = b(u^j),$$

and

$$\sum_j p_{ij} = 1.$$

From the definition of convex function and the properties of p_{ij} it follows immediately that

$$a \underset{2}{>} b \rightarrow a \underset{1}{>} b.$$

This note is devoted to the proof of

THEOREM: $$a \underset{1}{>} b \rightarrow a \underset{2}{>} b.$$

In the case where U is one-dimensional, the ordering given by these and other equivalent characterizations has been investigated or applied by I. Schur,[10] Muirhead,[6] Hardy, Littlewood, Polya,[2, 3] R. Rado,[3] Karamata,[4] Popoviciu,[8, 9] Weyl[12] and Shannon.[11] By way of Muirhead's Theorem a good many of the classical inequalities, for example, the relation between the arithmetic and geometric means can be made to follow. Weyl[13] and Polya[7] have recently considered the application of this set of ideas to eigenvalues of linear transformations. Recently this ordering has arisen in the work of C. E. Shannon[11] on prediction and entropy of printed English. Hardy, Littlewood, and Polya[3] established the equivalence of $\underset{1}{>}$ and $\underset{2}{>}$ for the case of measures consisting of a finite number of atoms, all of equal size. Some considerations in the theory of games led Bohnenblust, Shapley and Sherman, who were unaware of Hardy, Littlewood, and Polya's work, to investigate $\underset{1}{>}$ for the general U. Blackwell,[1] again unaware of Hardy, Littlewood, and Polya's work, suggested from considerations of sufficient statistics that $\underset{1}{>}$ and $\underset{2}{>}$ were equivalent. He proved the equivalence for one-dimensional U and general finite measures (no restriction on the spectrum).

Some notation in connection with linear spaces is useful at this point. Let E be a real linear space spanned by the n independent vectors $\{e_i \mid 1 \leq i \leq n\}$. Let $P = \{\sum_{1 \leq i \leq n} \alpha_i e_i \mid \alpha_i \geqq 0\} \subset E$ be a "positive cone." Let $\bar{E}$ be the conjugate space of E, i.e., the collection of real linear functionals $\bar{a}$ on E, where the value of a at $\bar{a}$ is given by $(\bar{a}, a)$. If A is a subset of real linear space then let $[A]$ be the closed linear subspace spanned by A.

Let $A^* = \{\bar{a} \mid \bar{a} \in \bar{E}, (\bar{a}, a) \geq 0 \text{ for each } a \in A\}$.

Let $A^\perp = \{\bar{a} \mid \bar{a} \in \bar{E}, (\bar{a}, a) = 0 \text{ for each } a \in A\}$.

Let $A^+ = A \cap P$. Let $\bar{e} \in \bar{E}$, where $(\bar{e}, e_i) = 1$ for each $i = 1, 2, \ldots, n$.

Suppose E is the real linear space generated by the measure with spectrum contained in given set $S = \{v_i \mid 1 \leq i \leq n\} \subset U$. E is generated by $\{e_i \mid 1 \leq i \leq n\}$, where e_i is the unit atomic measure concentrated at v_i. Let E_i represent the subspace of E generated by measures with centroid at v_i. It is of interest to characterize the set of all $a \in E$ such that $a \underset{1}{>} 0$, i.e., for each real convex φ, $\sum_i \varphi(v_i) a(v_i) \geqq 0$. To this end we note that if we define $CON \subset E$ by

$$CON = \bigcap \{((E_i^+)^* \cap e_i^\perp) + [\bar{c}] \mid 1 \leq i \leq n\},$$

then CON is a subset of $\bar{E}$ and is a representation of those functions on S which admit of a convex extension to U, i.e., to each $\bar{a} \in CON$ there corresponds a $\varphi_{\bar{a}} \mid S$ such that $(\bar{a}, e_i) = \varphi_{\bar{a}}(u_i)$ and $\varphi_{\bar{a}} \mid S$ admits of a convex extension to U. Then, for example, by the duality considerations in Weyl[12]

$$\begin{aligned} CON^* &= \sum \{((E_i^+)^* \cap e_i^\perp)^* \cap \bar{c}^\perp \mid 1 \leq i \leq n\} \\ &= \sum \{(E_i^+ + [e_i]) \cap \bar{c}^\perp \mid 1 \leq i \leq n\}. \end{aligned}$$

CON^* is of course the cone $\{a \mid a \in E, a > 0\}$.

If a and b are measures such that $a > b$ then $a\text{-}b \in CON^*$. If we wish to prove our theorem we can obviously confine ourselves to the case where $S_a \cap S_b$ is empty. Let $\mathcal{E}_a = [\{e_i \mid 1 \leq i \leq n_a\}]$ and $\mathcal{E}_b = [\{e^j \mid 1 \leq j \leq n_b\}]$. Our theorem may be regarded as an assertion about the structure of

$$(\mathcal{E}_a^+ + \mathcal{E}_b^-) \cap (CON^*).$$

In particular the assertion made by the theorem is

$$(\mathcal{E}_a^+ + \mathcal{E}_b^-) \cap (CON^*) = \sum_{1 \leq i \leq n_a} (\mathcal{E}_a^+ \cap (E_i^+ + [e_i]) \cap \bar{c}^\perp).$$

Obviously the right set is a subset of the left set.

From the structure of CON^* we know if $a \in CON^*$ then

$$a_i = \sum_{1 \leq i \leq n_a} a_i + \sum_{1 \leq j \leq n_b} a^j$$

where

$$\begin{aligned} a_i &= \alpha_i e_i + \sum_{1 \leq r \leq n_a} \alpha_{ir} e_r + \sum_{1 \leq s \leq n_b} \alpha_i^s e^s, \\ &\alpha_i \leq 0, \\ &\alpha_{ir} \geq 0,\ \alpha_i^s \geq 0,\ \alpha_{ii} = 0, \\ &\alpha_i + \sum_{1 \leq r \leq n_a} \alpha_{ir} + \sum_{1 \leq s \leq n_b} \alpha_i^s = 0, \\ &-\alpha_i u_i = \sum_{1 \leq r \leq n_a} \alpha_{ir} u_r + \sum_{1 \leq s \leq n_b} \alpha_i^s u^s, \end{aligned} \tag{1}$$

and

$$\begin{aligned} a^j &= \alpha^j e^j + \sum_{1 \leq r \leq n_a} \alpha^j{}_r u_r + \sum_{1 \leq s \leq n_b} \alpha^{js} u^s, \\ &\alpha^j \leq 0, \\ &\alpha^j{}_r \geq 0,\ \alpha^{js} \geq 0,\ \alpha^{jj} = 0, \\ &\alpha^j + \sum_{1 \leq r \leq n_a} \alpha^j{}_r + \sum_{1 \leq s \leq n_b} \alpha^{js} = 0, \\ &-\alpha^j u^j = \sum_{1 \leq r \leq n_a} \alpha^j{}_r u_r + \sum_{1 \leq s \leq n_b} \alpha^{js} u^s. \end{aligned} \tag{2}$$

What we should like to show is that

$$\alpha_i = 0,\ 1 \leq i \leq n_a$$

and

$$\alpha^{js} = 0,\ 1 \leq s \leq n_b.$$

If now we could show

LEMMA: *For each non-zero* $a\text{-}b \in (\mathcal{E}_a^+ + \mathcal{E}_b^-) \cap CON^*$ *there exists* i, $1 \leq i \leq n_a$, *and non-zero* $d \in \mathcal{E}_a^+ \cap (E_i^+ + [e_i]) \cap \bar{e}^\perp$ *such that* $a\text{-}b > d > 0$, then the theorem would readily follow.

Proof of Lemma: There are two alternatives: either (i) $a\text{-}b$ is on the boundary of CON^* or (ii) $a\text{-}b$ is in the interior of CON^*. In either event the proof involves an induction argument.

Case i. Here by Weyl,[12] or Krein and Rutman,[5] there exists a convex $\varphi \mid U$, non-linear on $Co(S)$, the least convex set containing S, such that

$$\sum_i \varphi(u_i)a(u_i) = \sum_j \varphi(u^j)b(u^j).$$

If we apply φ to both sides of (1) and (2) then we get

$$-\alpha_i\varphi(u_i) \leqq \sum_{1 \leq r \leq n_a} \alpha_{ir}\varphi(u_r) + \sum_{1 \leq s \leq n_b} \alpha_i{}^s\varphi(u^s) \tag{3}$$

and

$$-\alpha^j\varphi(u^j) \leqq \sum_{1 \leq r \leq n_a} \alpha^j{}_r\varphi(u_r) + \sum_{1 \leq s \leq n_b} \alpha^{js}\varphi(u^s). \tag{4}$$

Since

$$+\alpha_i + \sum_{1 \leq r \leq n_a} \alpha_{ri} + \sum_{1 \leq s \leq n_b} \alpha^s{}_i = a(u_i)$$

and

$$+\alpha^j + \sum_{1 \leq r \leq n_a} \alpha_r{}^j + \sum_{1 \leq s \leq n_b} \alpha^{sj} = -b(u^j)$$

we see by adding both sides of (3) and (4) that the sums result in an equality. Therefore each of the weak inequalities of (3) and (4) is an equality, i.e.,

$$-\alpha_i\varphi(u_i) = \sum_{1 \leq r \leq n_a} \alpha_{ir}\varphi(u_r) + \sum_{1 \leq s \leq n_b} \alpha_i{}^s\varphi(u^s)$$

and

$$-\alpha^j\varphi(u^j) = \sum_{1 \leq r \leq n_a} \alpha^j{}_r\varphi(u_r) + \sum_{1 \leq s \leq n_b} \alpha^{js}\varphi(u^s).$$

The last set of equations coupled with the convexity of φ and structure of E_i can be used to show that φ is linear over Co_i the least convex set containing u_i, those u_r such that $\alpha_{ir} > 0$ and those u^s such that $u_i{}^s > 0$. Likewise φ is linear over Co^j the least convex set containing u^j, those u_r such that $\alpha^j{}_r > 0$ and those u^s such that $\alpha^{js} > 0$. If φ is linear over two convex sets C_1 and C_2 which have a relative interior point of one in common, then φ is linear over $Co(C_1, C_2)$, the least convex set containing both C_1 and C_2. This follows from the case when C_1 and C_2 are line segments and in this case it can be verified in an elementary manner.

For each j, Co^j is non-empty. Moreover u^j is a relative interior point of Co^j. If any of the Co_i is non-empty then u_i is a relative interior point of Co_i. For each j let $L^j = \{u^j\}$. Let $R^j =$ the set of vectors which have non-zero coefficients in the expansion (2) for $-\alpha^j u^j$. We make the analogous definition for L_i and R_i except that the sets are defined to be empty when Co_i is empty. With each Co^j, we associate CO^j the maximal convex subset of $Co(S)$ containing Co^j such that φ is linear over CO^j. Of course $CO^j \supset Co^j$. Analogous remarks apply to CO_i for such i that Co_i is non-empty. We define $L(CO^j) = \bigcup \{L^j \mid Co^j \subset CO^j\} \cup \bigcup \{L_i \mid Co_i \subset CO^j\}$. Analogous definitions are given to $L(CO_i)$, $R(CO^j)$ and $R(CO_i)$. For any CO set, $L(CO)$ has no element in common with any $L(CO)$ or $R(CO)$ for a different CO. Two different CO's may have common elements in their $R(CO)$'s, but only if these common elements are not represented in any $L(CO)$. The common elements, if they exist, must be elements of S_a.

Since φ is non-linear on $Co(S)$, there must be at least two different CO's. Let us call them $CO(1)$ and $CO(2)$. On account of the remarks made about $L(CO)$ and $R(CO)$ we see that $L(CO(1)) \cup R(CO(1))$ is a proper subset of S. Let us consider the collection of a_i such that $Co_i \subset CO(1)$ and a^j such that $Co^j \subset CO$. The sum of these a_i and a^j is a measure whose spectrum is contained in $L(CO(1)) \cup R(CO(1))$, a proper subset of S. This subsidiary sum is contained in $(\mathcal{E}_a{}^+ + \mathcal{E}_b{}^-) \cap CON^*$. Analogous remarks hold for the other CO's. If now our lemma were true for spectrum of cardinal number smaller than $n_a + n_b$, each of the subsidiary sums could be decomposed in the manner prescribed by the lemma so that the full sum could be decomposed.

Case ii. a-b is interior to CON^*. There is a neighborhood G (relative to CON^*) of 0 of non-zero elements g such that $a\text{-}b > g > 0$. If $CON^* \subset \mathcal{E}_a{}^+ + \mathcal{E}_b{}^-$ then the lemma is trivially true. If CON^* is not a subset of $\mathcal{E}_a{}^+ + \mathcal{E}_b{}^-$, then there exist $g \in G$ such that g is not an element of $\mathcal{E}_a{}^+ + \mathcal{E}_b{}^-$. Since CON^* contains no one-dimensional subspace the line segment joining g and a-b intersects the boundary of $\mathcal{E}_a{}^+ + \mathcal{E}_b{}^-$ at a non-zero element h. Then $a\text{-}b > h > 0$. Since $h \in$ boundary of $\mathcal{E}_a{}^+ + \mathcal{E}_b{}^-$ the spectrum of h is a proper subset of S. The validity of the lemma for spectrum of lower

cardinal number would imply the lemma in this case.

Low Dimensional Case. The requirements that $a\text{-}b(\neq 0) \underset{1}{>} 0$ and $a\text{-}b \in \mathcal{E}_a^+ + \mathcal{E}_b^-$ imply that $n_a + n_b \geqq 3$. The sum $n_a + n_b = 3$ and the requirement that $a\text{-}b \underset{1}{>} 0$ imply from the arguments in case i and case ii that $CON^* \subset \mathcal{E}_a^+ + \mathcal{E}_b^-$, otherwise there would be an $a\text{-}b$ with spectrum of lower cardinal number and satisfying the conditions in the hypothesis of the lemma. With this the lemma and theorem are proved.

It would be of interest to get a vector analog to $\underset{1}{>}$ as is implicitly given in the one-dimensional case by Muirhead's Theorem.[3]

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RADIATION CAPACITY

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Capacity is a classical notion in the theory of electrostatic fields. This note deals with its analog for radiating fields (unquantized!). The static theory will be reproduced here briefly in a form evincing the points where it carries over to radiating fields or where essential modifications are needed.

1. The static theory in Euclidean 3-space is governed by Newton's potential

$$Q(pp') = 1/2\pi r \tag{1}$$

where $r = r(pp')$ is the distance of any two points p, p'. Suppose the space is divided into two complementary parts, the connected "exterior" W and the bounded "interior" V. The latter may exist of a finite number h of connected components V_i, the conductors. Their surfaces Ω_i constitute the common boundary Ω of V and W, which we assume to be smooth, i.e., the unit vector of the external normal at the variable point s of Ω is not only a continuous function of s, but also satisfies a Hölder condition. The external boundary problem asks for a harmonic function $u(p)$ in W, $\Delta u = 0$, tending to zero uniformly in all directions if p approaches infinity, and assuming given boundary values $\gamma(s)$ on Ω. Since uniqueness holds, i.e., since $\gamma(s) = 0$ on Ω implies $u(p) = 0$ in W, one expects that the problem has a unique solution whatever the given function $\gamma(s)$ on Ω. One tries to obtain it in the form of the potential of a dipole layer,

$$u(p) = \int_\Omega (\partial Q(ps')/\partial n_{s'}) \cdot \mu(s')ds'. \qquad [D]$$

Here $\mu(s)$ is an unknown continuous function on Ω, and $\partial/\partial n$ stands for differentiation along the external normal. Integration with respect to the variable point s or s' of Ω always extends over the whole of Ω; ds or ds' is the area element. The condition that $u(p)$ on the external side of Ω assumes the values $\gamma(s)$ leads to the integral equation

$$\mu(s) + \int K(ss')\mu(s')ds' = \gamma(s), \quad \text{briefly } (E + K)\mu = \gamma \tag{2}$$

for the layer density $\mu(s)$. The Hölder condition keeps the singularity of its kernel

$$K(s, s') = \partial Q(ss')/\partial n_{s'} \tag{3}$$

for $s = s'$ so low that Fredholm's theory is applicable. The diffculty stems from the fact that the corresponding homogeneous equation

$$(E + K)\varphi = 0 \tag{4}$$

and hence also the homogeneous equation with the transposed kernel,

$$\eta(E + K) = 0 \qquad \text{or} \qquad \eta(s') + \int \eta(s)K(ss')\,ds = 0, \quad (4')$$

each have an h-dimensional manifold Φ and H of solutions. (Our form of writing emulates the matrix calculus as if K were a square matrix and the values of φ arranged in a column and those of η in a row.) The solutions of (4) are the functions $\varphi(s)$ which assume arbitrary constant values q_i on each of the conductor surfaces Ω_i. A solution of (4') gives rise to a field of simple layer

$$v(p) = \int Q(ps') \cdot \eta(s')ds', \qquad (5)$$

harmonic both in V and W, continuous even on the boundary Ω while the normal derivative $\partial v/\partial n$ vanishes on the inner side of Ω, and assumes the value $-2\eta(s)$ at the point s of the outer side. The vanishing of the inner derivative $\partial v/\partial n$ implies that v has a constant value q_i in each conductor V_i including its surface: (5) is the general solution of the conductor problem. Thus we have as our

First fact: The formula

$$\varphi(s) = \int Q(ss') \cdot \eta(s')\,ds'$$

establishes a linear mapping M, $\eta \to \varphi = M\eta$, of H into Φ.

From the uniqueness theorem there follows the

Second fact: This mapping is non-singular, i.e., $M\eta = 0$ implies $\eta = 0$.

Hence M is a one-to-one mapping of H upon Φ. Two elements η of H and φ of Φ have a product

$$(\eta\varphi) = \int \eta(s)\varphi(s)\,ds.$$

Our

Third fact is the law of symmetry $C(\eta, \eta^*) = C(\eta^*, \eta)$ for the bilinear form

$$C(\eta, \eta^*) = (\eta, M\eta^*)$$

of two arbitrary elements η, η^* of H.

Indeed

$$(\eta, M\eta^*) = \int\int \eta(s)Q(ss')\eta^*(s')\,ds'\,ds.$$

Fourth fact: The symmetric bilinear form $C(\eta, \eta^*)$ is non-singular; i.e., if an element η^* of H satisfies the condition $C(\eta, \eta^*) = 0$ for every $\eta \in$ H then $\eta^* = 0$.

This follows since $C(\eta, \eta)$ is nothing but the Dirichlet integral of (5) extended over the whole space and therefore positive unless $v = 0$, $\eta = 0$.

One may choose the basis φ_i for Φ such that $\varphi_i(s) = 1$ on Ω_i, $= 0$ on all other Ω_j $(j \neq i)$. Form the linear combination $\eta = q_1\eta_1 + \ldots + q_h\eta_h$ of the

$\eta_i = M^{-1}\varphi_i$ with constant coefficients q_i: the solution (5) of the conductor problem with this η corresponds to the values q_i for the potentials of the several conductors V_i. The total charge e_i of V_i is given by $2(\eta\varphi_i)$, and hence potentials and charges are connected by the linear relations

$$e_i = 2\cdot\sum_j c_{ji}q_j \qquad \{c_{ji} = (\eta_j\varphi_i)\}.$$

Thus c_{ji}, the symmetric coefficients of the quadratic form $C(\eta\eta) = \sum c_{ji}q_jq_i$, are the (halved) capacity coefficients.

From the fourth fact follows the unique existence of an element $\eta^* \epsilon$ H such that $\varphi^* = M\eta^*$ satisfies the condition $(\eta\varphi^*) = (\eta\gamma)$ for every $\eta \epsilon$ H. For the boundary values $\gamma^* = \gamma - \varphi^*$ one can therefore construct a potential $u^*(p)$ by means of a suitable double layer $\mu^*(s)$. Adding to it the potential $v^*(p)$ of the simple layer η^*, chosen according to the above construction from the special manifold H, one obtains the desired field $u(p)$.

2. Let us now pass from static potential to scalar radiation. Given a positive constant k, we are concerned with scalar fields $u(p)$ in W satisfying the equation $\Delta u + k^2u = 0$ in W and the condition of outgoing radiation:

$$R(\partial u/\partial n + iku)_{\text{on } \Sigma_R} \to 0 \qquad \text{with } R \to \infty$$

(admissible fields). i is the imaginary unit, Σ_R the sphere of radius R around the origin. The main question is: can one assign arbitrary boundary values on Ω for such a field?

The place of (1) is now taken by

$$Q(pp') = e^{-ikr}/2\pi r. \tag{6}$$

The uniqueness theorem has been established by W. Magnus and F. Rellich in 1943.[1]

The formula (2) (with the new Q) gives an admissible field in W with the boundary values

$$(E + K)\mu = \gamma \tag{2}$$

where $K(s, s')$ is the kernel (3). What stands in the way of solving the external boundary problem in this fashion is the circumstance that the homogeneous equations

$$(E + K)\varphi = 0 \qquad \text{and} \qquad \eta(E + K) = 0$$

may have non-vanishing solutions. They form linear manifolds Φ and H of the same number h of dimensions, and the equation (2) is solvable for a given γ only if γ is orthogonal to all the elements η of H, $(\eta\gamma) = 0$. (The number h has now nothing to do with the number of connected components of V.)

The external boundary problem for scalar and also for electromagnetic radiation has recently been treated by W. K. Saunders.[2] He overcame the

difficulty just mentioned by an ingenious but highly artificial device which he ascribes to a suggestion by H. Lewy. I asked myself whether the construction of capacity coefficients for radiating fields would not provide a better and more natural way out. Checking the above four facts I found that the first, second and third hold good, but since the argument by means of the definite Dirichlet integral fails there is no reason to expect the universal validity of the fourth fact. Nor is this supported by the general theory of integral equations—at least not unless one extends the manifold Φ of the eigen-functions to that of the "principal functions" which the theory of elementary divisors deals with.

Let $K(ss')$ be an arbitrary kernel regular enough for the applicability of Fredholm's theory, and let A be the operator $E + K$. We form the finite-dimensional manifolds Φ_0, Φ_1, Φ_2, . . . of the solutions φ of the successive equations

$$\varphi = 0, \qquad A\varphi = 0, \qquad A^2\varphi = 0, \ldots .$$

The sequence $\Phi_0 \subset \Phi_1 \subset \Phi_2 \subset \ldots$ becomes stable after a number l of steps: $\Phi_l = \Phi_{l+1} = \Phi_{l+2} = \ldots$ while Φ_{l-1} is still a proper part of Φ_l. The manifold H_m of the solutions η of $\eta A^m = 0$ is of the same dimensionality h_m as Φ_m. Set $h_1 = h$, $\Phi_1 = \Phi$, $H_1 = H$ and $h_l = n$. According to the general theory an η of the n-dimensional manifold H_l vanishes if it satisfies $(\eta\varphi) = 0$ for all $\varphi \in \Phi_l$: the bilinear form $(\eta\varphi)$ for $\eta \in H_l$, $\varphi \in \Phi_l$ is non-singular.

Use the abbreviations $A\varphi = \varphi'$, $\eta A = \eta'$, and observe the simple rule

$$(\eta'\varphi) = (\eta\varphi').$$

The elements of the form φ' $(\varphi \in \Phi_l)$ constitute a manifold Φ_l' of dimensionality $n - h$. Hence let $\varphi_1^*, \ldots, \varphi_h^*$ be h elements of Φ_l that are linearly independent mod. Φ'_l and $\eta_1, \ldots, \eta_h$ a basis of H. I maintain that the square matrix

$$d_{ij} = (\eta_i\varphi_j^*) \qquad (i, j = 1, \ldots, h) \tag{7}$$

is non-singular, $\det d_{ij} \neq 0$. Indeed every element $\eta \in H$ is orthogonal to the elements φ' of Φ'_l because $(\eta\varphi') = (\eta'\varphi) = 0$; hence if it is also orthogonal to $\varphi_1^*, \ldots, \varphi_h^*$ it is orthogonal to all $\varphi \in \Phi_l$ and therefore zero.

With this in mind we return to the kernel (3) derived from (6) and again examine the four facts, now for Φ_l and H_l instead of Φ_1 and H_1. Does M map H_l into Φ_l? The answer is yes. For any $\eta \in H_l$ form $\varphi = M\eta$ and again set $\eta' = \eta(E + K) = \eta A$. One infers from Green's formula that for points p in W the equation holds

$$\int (\partial Q(ps')/\partial n_{s'}) \cdot \varphi(s')\, ds' = \int Q(ps')\eta'(s')\, ds'. \tag{8}$$

Letting p tend to a point s on Ω from the exterior one gets

$$(E + K)\varphi = M\eta';$$

in other words: if M carries η into φ it carries $\eta' = \eta A$ into $\varphi' = A\varphi$. This proves even considerably more than the "first fact."

Fact 2 holds good for the mapping M of H_l upon Φ_l because of Rellich's uniqueness theorem. Fact 3 is trivial, and fact 4 is identical with the universally valid statement that $(\eta\varphi)$ is non-singular for $\eta \in \mathrm{H}_l$, $\varphi \in \Phi_l$. Thus the external problem can be solved in the manner outlined for the static case if only one uses the manifolds H_l and Φ_l instead of H and Φ.

Still there remains something to be desired. For we know that the "potential" $u(p)$, equation [D], of a suitable double layer $\mu(s)$ solves the problem for given boundary values $\gamma = \gamma(s)$ if γ is orthogonal to the elements η of $\mathrm{H} = \mathrm{H}_1$; it need not be orthogonal to all the elements of H_l! But for every element η' of H_l' the formula (8) shows that the potential of the simple layer η' is identical with that of the double layer $\varphi = M\eta$. This remark clarifies the situation completely and suggests the following procedure. One chooses $\eta_1^*(s), \ldots, \eta_h^*(s)$ as h elements of H_l that are linearly independent modulo H_l'. They are mapped by M into h elements $\varphi_1^*, \ldots, \varphi_h^*$ of Φ_l that are linearly independent mod. Φ_l'. If $\eta_1, \ldots, \eta_h$ is a basis of H the determinant of the coefficients (7) does not vanish. We can therefore choose h constants α_j such that

$$\gamma^*(s) = \gamma(s) - \sum_{j=1}^{h} \alpha_j \varphi_j^*(s)$$

is orthogonal to $\eta_1, \ldots, \eta_h$. For the boundary values $\gamma^*(s)$ we have a solution in form of the potential of a double layer; to it we add the linear combination $\sum_j \alpha_j v_j^*(p)$ of the potentials $v_j^*(p)$ of the simple layers $\eta_j^*(s)$. These are exactly those potentials of simple layers chosen from the manifold H_l which cannot be carried over into double layer potentials.

The analysis may be pursued to finer details. It is not without interest that here we have a case in mathematical physics where proof of non-degeneracy of a quadratic form is not based on its definite character, and where one can cope successfully with the complications of elementary divisors of higher degree than 1.

3. The best choice of surface layers for the treatment of electromagnetic radiation is set forth in §3 of an old paper of mine[3] on the eigen-frequencies of elastic bodies. It superseded an earlier paper[4]. Since, unfortunately, Mr. Saunders followed the bad example set by me in the latter I propose to come back to the external boundary problem of outgoing electromagnetic waves in a more systematic paper on radiation capacity.

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ELECTRON-NEUTRON INTERACTION

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I. Introduction.—An explanation of the approximate value of the electron-neutron (*e-n*) interaction has been advanced by Foldy.[1] He points out that the approximate magnitude of the experimentally observed effect can be understood in terms of an intrinsic magnetic moment of the neutron and that previous explanations in terms of meson theories are probably significant only to the degree to which they are consistent with the more directly phenomenologic explanation in terms of the intrinsic moment. The effect under discussion is independent of spin direction and is for this reason somewhat less directly connected with a simple picture of the action of a magnetic moment than the splitting of energy levels caused by differences in relative spin orientations. The effect on the energy of a system caused by an interaction of an intrinsic moment with an electric field has been noted previously in connection with a study of the intrinsic magnetic moment of the electron.[2] It appeared in that connection as a contribution to the Lamb Shift of the order of 70 mc/sec in a total of 1062 mc/sec and it was impossible to separate this effect from corrections to the theoretical values for the Lamb Shift arising from other causes. The calculation of the effect in relation to the hydrogen spectrum did not lead therefore to comparison with experiment. Since on the other hand it has been explicitly calculated the results can be used to indicate general features of the expected *e-n* interaction. In particular one observes that the interaction of the moment with the electric field is only about $-1/3$ for particles in a $p_{1/2}$ state of what it is for s states and that it is only partially of the local type describable by a term in $\delta(\mathbf{r}_e - \mathbf{r}_n)$. In view of the new interest in the results expected as consequences of such an interaction it appears desirable to record a few simple considerations regarding the nature of its origin, which can be understood without the employment of the canonical transformation of Foldy and Wouthuysen.[3]

II. The Interaction Energy.—The equation satisfied by the wave function is taken to be

$$(\hbar/i)(\partial\psi/\partial t) + H\psi = 0 \tag{1}$$

$$H = -eA_0 - c(\boldsymbol{\pi}\boldsymbol{\alpha}) - \rho_3 mc^2 + \mu[\rho_3(\mathcal{H}\sigma) - \rho_2(\mathcal{E}\sigma)] \tag{1.1}$$

where

$$\boldsymbol{\pi} = \mathbf{p} + e\mathbf{A}/c \tag{1.2}$$

is the operator representing the mechanical momentum while

$$\mathbf{p} = (\hbar/i)\nabla$$

and A_0, $\mathbf{A}$ denote the scalar and vector potential. The matrices $(\boldsymbol{\alpha}, \rho_3)$ form the four Dirac alphas while ρ_1, ρ_2, ρ_3 are Dirac's three matrices satisfying $\rho_1\rho_2 = -\rho_2\rho_1 = i\rho_3$, and two more relations obtainable from the one just written by cylic interchange. The notation is that of Dirac's first papers, the charge on the particle being denoted by $-e$. One may write

$$H = H_0 + H'$$

where

$$H' = \mu\{\rho_3(\mathfrak{H}\boldsymbol{\sigma}) - \rho_2(\mathfrak{E}\boldsymbol{\sigma})\}.$$

The addition of H' to H_0 does not alter the equations of motion

$$d\mathbf{r}/dt = (i/\hbar)[H, \mathbf{r}] = -c\boldsymbol{\alpha} \tag{2}$$

and the relation of $d\boldsymbol{\pi}/dt$ to the Lorentz force operator with $\boldsymbol{\alpha}$ taking place of the classical $-d\mathbf{r}/c\,dt$ is affected, therefore, only through the addition of

$$\delta(d\pi_x/dt) = (i/\hbar)[H', p_x] = \mu\{-\rho_3(\sigma\partial\mathfrak{H}/\partial x) + \rho_2(\sigma\partial\mathfrak{E}/\partial x)\}. \tag{2.1}$$

The operator on the right side of this equation gives, therefore, the addition to the force on the particle. Its expectation value at low velocities is the same as the expectation value of

$$\mu(\partial/\partial x)(\mathfrak{H}\boldsymbol{\sigma}),$$

since for low velocities one may use a representation of Dirac matrices in which

$$\rho_3 = \begin{pmatrix} +1 & 0 & 0 & 0 \\ 0 & +1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 \end{pmatrix}$$

and ψ_3, ψ_4 are large in comparison with ψ_1, ψ_2. For curl $\mathfrak{H} = 0$ the addition to $d(\delta\pi_x)/dt$ is

$$\mu(\sigma_x\partial\mathfrak{H}_x/\partial x + \sigma_y\partial\mathfrak{H}_x/\partial y + \sigma_z\partial\mathfrak{H}_x/\partial z) \tag{2.2}$$

and this expression has the same form as the classical expression for the x component of the force on a magnetic doublet $\mu\boldsymbol{\sigma}$ supposed to be at rest. The expectation value of H' in the non-relativistic limit is

$$\langle H'\rangle_{\mathrm{NR}} = -\mu(\langle\mathfrak{H}\cdot\boldsymbol{\sigma}\rangle) \tag{2.3}$$

in agreement with the non-relativistic formula for the force.

It is of interest to examine the consequences of the introduction of H' in the special case of plane waves. The spin direction in the rest system

of the particle will be taken to be along the z axis. This choice of orientation is established by taking $\psi_4 = 0$. A simple calculation gives

$$\langle -H'/\mu \rangle = \mathfrak{H}_z - p_z(\mathbf{p}\mathfrak{H})/[p_0(p_0 + mc)] + [\mathcal{E} \times \mathbf{p}]_z/p_0. \quad (3)$$

Here

$$p_0 = E/c$$

and all quantities are now ordinary numbers rather than operators. The expectation value of the force is

$$\langle F_x/\mu \rangle = \partial\mathfrak{H}_z/\partial x - p_z(\mathbf{p}\partial\mathfrak{H}/\partial x)/[p_0(p_0 + mc)] + [(\partial\mathcal{E}/\partial x) \times \mathbf{p}]_z/p_0. \quad (3.1)$$

This expression has the structure of the force on a magnetic doublet of unit strength oriented along the z axis and moving with velocity

$$\mathbf{v} = \mathbf{p}/p_0.$$

One can visualize the transformations[4] by considering a doublet having strength 1 in its rest system with doublet axis along OZ in the rest system. In this system the properties of the classical doublet can be reproduced by evaluating corresponding properties of a system composed of two magnetic poles, of total pole strength zero. The change in the magnetic doublet strength is obtainable, therefore, by a consideration of the effect of the Lorentz transformation on the displacement vector of the positive with respect to the negative pole. In the rest system a unit vector along the z axis is resolved along the line of $\mathbf{v}$ and in a plane perpendicular to $\mathbf{v}$. Only the former is changed by Lorentz contraction. The change in the strength of the unit doublet is therefore

$$\{[1 - (v^2/c^2)]^{1/2} - 1\}(v_z/v)(\mathbf{v}/v). \quad (3.2)$$

The change in magnetic energy obtained from the wave equation is

$$+p_z(\mathbf{p}\mathfrak{H})/[p_0(p_0 + mc)] = [-1 + (mc/p_0)](v_z/v)(\mathbf{v}\mathfrak{H}/v) \quad (3.3)$$

and the bracket in the last formula is the value of the quantity in curly braces in the preceding one. The classical model and the Pauli intrinsic spin equation are seen to agree in the case of plane waves so far as terms in magnetic energy are concerned.

If the doublet were electric its effective magnetic moment due to translation could be obtained by decomposition into doublets parallel and perpendicular to the line of motion. Only the perpendicular component contributes to the motional magnetic moment and it has the value $[\mathbf{1}_z \times (\mathbf{v}/c)]$. In a magnetic field its energy would be $-\mathfrak{H}\cdot[\mathbf{1}_z \times \mathbf{v}]/c = [\mathfrak{H} \times (\mathbf{v}/c)]\cdot\mathbf{1}_z$. The symmetry of Maxwell's equations in electric and magnetic fields with attention to the change in sign present in motional

effects shows that for a magnetic doublet the mutual energy with an electric field is

$$-[\mathcal{E} \times (\mathbf{v}/c)]\cdot \mathbf{1}_z, \tag{3.4}$$

in agreement with the expression derived from the wave equation. It is seen again therefore[4] that Pauli's covariant interaction corresponds to a magnetic doublet of permanent strength μ in the notation used above.

III. The Non-Relativistic Approximation.—The effect of μ in the non-relativistic approximation can be understood without the aid of the special canonical transformation of Foldy and Wouthuysen[3] the properties of which have not as yet been sufficiently tested to make sure that it yields results equivalent to more familiar forms of theory. Employing the well-known representation of Dirac's matrices in which

$$\begin{pmatrix} \psi_3 \\ \psi_4 \end{pmatrix} = \Psi \tag{4}$$

are much larger at small velocities than

$$\begin{pmatrix} \psi_1 \\ \psi_2 \end{pmatrix} = \Phi, \tag{4.1}$$

the wave equation for fixed energy is

$$[E + eA_0 + mc^2 - \mu(\mathcal{H}\cdot\sigma)]\Phi + [c\boldsymbol{\pi}\boldsymbol{\sigma} - i\mu\,(\mathcal{E}\sigma)]\Psi = 0 \tag{4.2}$$

$$[E + eA_0 - mc^2 + \mu(\mathcal{H}\cdot\sigma)]\Psi + [c\boldsymbol{\pi}\boldsymbol{\sigma} + i\mu\,(\mathcal{E}\sigma)]\Phi = 0.$$

It follows that for low velocities

$$\Phi = Q\Psi, \tag{4.3}$$

$$Q \cong -[(c\boldsymbol{\pi} - i\mu\mathcal{E})\cdot\boldsymbol{\sigma}]/(2mc^2) \cong -(\boldsymbol{\pi}\cdot\boldsymbol{\sigma})/(2mc), \tag{4.4}$$

the plan of the calculation being to neglect terms non-linear in μ and in the coefficient of μ to keep only terms linear in the field strengths. Substituting by means of Eqs. (4.3), (4.4) the value of Φ into the second of the two Eqs. (4.2) connecting Φ and Ψ one obtains an addition to the two component operator for E arising from Φ, *viz.*

$$H' = (1/2mc^2)[c(\boldsymbol{\pi}\sigma) + i\mu(\mathcal{E}\boldsymbol{\sigma})][c(\boldsymbol{\pi}\boldsymbol{\sigma}) - i\mu(\mathcal{E}\boldsymbol{\sigma})].$$

The part of H' containing only the first power of μ is

$$H_1' = (i\mu/2mc)[(\mathcal{E}c), (\mathbf{p}\boldsymbol{\sigma})],$$

which is readily seen to be

$$H_1' = (\mu/2mc)\{-\hbar\, div\, \mathcal{E} + [\mathbf{p} \times \mathcal{E}]\cdot\boldsymbol{\sigma} - [\mathcal{E} \times \mathbf{p}]\cdot\boldsymbol{\sigma}\}. \tag{4.5}$$

This addition to the energy operator is identical with that calculated by

Foldy[1] except for a difference in notation. In the formula just written the magnetic moment μ has the dimensions of charge times distance.

The occurrence of a term in the expression for the energy does not show in itself that the term may be considered in the two-component equation as an addition to the Hamiltonian. One might argue that one should expect an equivalence of additions to the energy to additions to an effective two-component Hamiltonian because the general time dependent wave function may be expressed as a superposition of time independent factors corresponding to a definite energy E multiplied by $exp\ (-iEt/\hbar)$ and that a term such as H_1' will affect $\partial\Psi/\partial t$ in the same way as though one were dealing with a two component Hamiltonian. On the other hand it is only $\partial\Psi/\partial t$ expressed in terms of $H\Psi$ that matters in the usual reasoning introducing quantum mechanical equations of motion. This argument may perhaps be established more rigorously. It has an obvious weakness regarding an insufficiently detailed consideration of the possibility of representing $\partial\Psi/\partial t$ by using only a limited range of values of E. It is satisfying therefore to verify directly that the addition of H_1' to the two-component Hamiltonian is consistent with the original relativistic equation regarding the expectation value of $d\boldsymbol{\pi}/dt$.

In fact expressing Φ by means of Eq. (4.4) and employing

$$Q^* \cong -[(c\boldsymbol{\pi} + i\mu\mathcal{E})\cdot\boldsymbol{\sigma}]/(2mc^2) \tag{4.6}$$

one has on account of Eq. (2.1) a contribution to the expectation value of the force arising from H' as

$$\begin{aligned}\langle \delta F_x \rangle = {} & -\mu(\Psi,\ \{Q^*[\partial(\mathcal{H}\cdot\boldsymbol{\sigma})/\partial x]Q - \partial(\mathcal{H}\sigma)/\partial x\}\Psi) - \\ & -i\mu(\Psi,\ \{Q^*[\partial(\mathcal{E}\cdot\boldsymbol{\sigma})/\partial x] - [\partial(\mathcal{E}\cdot\boldsymbol{\sigma})/\partial x]Q\}\Psi).\end{aligned} \tag{5}$$

The partial differentiations apply here to $\mathcal{E}$ and $\mathcal{H}$. One may neglect the first term in $\mathcal{H}$ on account of the combined occurrence of Q and Q^* which results in this term being of order $(v/c)^2$ of the next term which also contains $\mathcal{H}$ and which corresponds to the elementary picture of magnetic doublet $\mu\boldsymbol{\sigma}$. The last two terms in Eq. (5) can be combined and linearized in $\boldsymbol{\sigma}$. It is thus found that in calculations with two-component wave functions in the non-relativistic approximation one may use the operator

$$\delta f_x = \mu[\partial(\mathcal{H}\cdot\boldsymbol{\sigma})/\partial x] - \partial H_1'/\partial x, \tag{6}$$

where it is again understood that $\partial/\partial x$ applies only to $\mathcal{E}$ and $\mathcal{H}$. The force operator of the two-component equation is here distinguished from the original operator through the employment of the lower case character f. In accordance with expectation the operator H_1' plays the role of a potential energy in calculations with a two-component wave function. The extra terms contained in $-\partial H_1'/\partial x$ are seen to have physical meaning

regarding the deflection of the centroid of a wave packet first emphasized by Ehrenfest which is an immediate consequence of Eq. (1.1).[5]

IV. Relation to Relativistic Calculations.—An effect of the type under discussion has been calculated[2] in connection with the problem of the intrinsic magnetic moment of the electron which was called μ_e in the reference quoted. The terms corresponding to the interaction of μ_e with the electric field of the nucleus were collected in the quantity H'''. For an electrostatic potential

$$Zev(r) = V(r) = A_0(r) \tag{7}$$

the expectation value of H''' was found to be

$$\langle H''' \rangle = 2\mu_e Ze \int_0^\infty (-v'r^2)fg\, dr, \tag{7.1}$$

where $v' = dv/dr$ so that for concentrated charge distributions $-v'r^2 = 1$. The functions f, g are the radial functions of Dirac's equation, the convention regarding relative signs of f and g being that of Eq. (1.6) of the reference. The normalization is such that

$$\int_0^\infty (f^2 + g^2)r^2\, dr = 1. \tag{7.1'}$$

According to Eq. (3.3) of the reference the non-relativistic approximation for a Coulomb field is

$$\langle H''' \rangle \cong 2Z\mu_0\mu_e[-2\pi\psi_S{}^2(0) + (1 + k)\langle r^{-3}\rangle(1 - \delta_{L,0})] \tag{7.2}$$

where k is the Dirac-Sommerfeld quantum number which has the values $-1, 1, -2, 2, \ldots$ for $s_{1/2}$, $p_{1/2}$, $p_{3/2}$, $\ldots$ terms respectively. For the same principal quantum number n the ratio of $\langle H''' \rangle$ for any k and L to that for $L = 0$ is according to Eq. (6.1) of the reference

$$\langle H''' \rangle / \langle H''' \rangle_s \cong -1/[k(2L + 1)]. \tag{7.3}$$

Here L is the azimuthal quantum number. The radial function f is small compared to g in the non-relativistic approximation and is obtainable from g as

$$f = c\hbar(E + eA_0 + mc^2)^{-1}[dg/dr + (1 + k)g/r], \tag{7.4}$$

which may be approximated by

$$f \cong (\hbar/2mc)[dg/dr + (1 + k)g/r]. \tag{7.4'}$$

For s terms one has from Eq. (7.1) employing the approximation of Eq. (7.4') and the notation of Eq. (7)

$$\langle H''' \rangle_s \cong (\mu_e\hbar/mc) \int_0^\infty (-r^2 dV/dr)g(dg/dr)\, dr. \tag{8}$$

It is supposed that V is non-singular at $r = 0$. Partial integration yields therefore

$$\langle H''' \rangle_s \cong [\mu_e\hbar/(2mc)] \int_0^\infty r^2 g^2(\Delta V)\, dr. \tag{8.1}$$

Taking into account the relation $\Delta V = -div\ \mathcal{E}$ this contribution to the expectation value of the energy is seen to be the same as that of the first term in the curly braces of Eq. (4.5).

For a point charge of magnitude Ze one may make the replacement

$$\Delta V = -4\pi Ze\delta(\mathbf{r}), \tag{8.2}$$

where δ is the three-dimensional Dirac delta function. The integral in Eq. (8.1) becomes therefore

$$-Zeg^2(0) = -4\pi Ze\psi_S{}^2(0) = \int_0^\infty r^2 g^2(\Delta V)\, dr, \tag{8.3}$$

the last equation following from the fact that in the non-relativistic approximation $\int_0^\infty g^2 r^2\, dr = 1$, while $\int_0^\infty \psi_S{}^2 r^2\, dr = 1/(4\pi)$ so that $g^2 = 4\pi\psi_S{}^2$. Combining Eqs. (8.1), (8.2), (8.3) and introducing $\mu_0 = e\hbar/(2mc)$ one has

$$\langle H''' \rangle_s = -4\pi Z\mu_0\mu_e\psi_S{}^2(0), \tag{8.4}$$

in agreement with Eq. (7.2). The latter, as well as its consequence, Eq. (7.3), show that there is, in general, an additional interaction which does not depend for its existence on $\psi^2(0)$. Such an interaction is of a non-local character. Its origin is the interaction of the electric doublet produced by the motion of the magnetic doublet with the electric field of the nucleus. The spin dependent terms correspond to the usual spin-orbit interaction and can be visualized as the energy of the electric moment $[(\mathbf{p}/m) \times \mu\sigma]$ with the electric field $\mathcal{E}$. This effect has been discussed in connection with Eq. (3.4).

According to Eq. (7.1) the origin of the term in $\psi_S{}^2(0)$ is similar to that of a similar term in Fermi's approximation for the hyperfine structure of s terms. Replacing the integral in Eq. (7.1) one has, apart from a constant factor, the product of the radial component of the electric field and of fg. The approximation for f breaks down at $r = e^2/mc^2$ but the contributions to the integral from such distances are small. Replacement of f by its non-relativistic approximation is justifiable within the limits familiar from hyperfine structure theory. The identity of the approximations involved can be seen by comparing Eq. (1.93) with Eq. (2.6) of the paper quoted.

V. Applicability to the Neutron.—According to Foldy the origin of the electron-neutron interaction observed in recent experiments is probably the spin independent contribution to the non-relativistic two-component Hamiltonian. This contribution is

$$H_1'' = -(\mu\hbar/2Mc)div\ \mathcal{E} \tag{9}$$

as follows from Eq. (4.5) on replacing the particle mass m by the neutron mass M. He finds good agreement with the experimental values and points

out that calculations using meson theory either agree with H_1'' or else contain omissions which could bring about agreement. The degree to which a phenomenologic treatment of the neutron may be expected to determine its interaction with an external electromagnetic field is of interest. A related question is whether the electron interacts with mesons and the bare neutron only in virtue of the electron's electromagnetic field. The second question probably involves too many unknowns to make its discussion practical. An answer to it probably can be made only by experiment. A brief discussion of the first question appears to be justifiable.

If one could assume the validity of Eq. (1.1) the whole discussion would be reduced to a calculation. Eq. (1.1) is, of course, only an approximation. It can hardly be expected to apply if the wave length of the electromagnetic field is much smaller than e^2/mc^2. Similarly if the origin of μ were to be similar to an atomic system showing a quadratic Zeeman effect the equation would not apply. Since there are these obvious possibilities of invalidating the applicability of the starting point, it appears worth while to attempt to point out characteristic features of the hypothetical system called the neutron which make the Pauli type equation applicable.

The interaction energy of the electromagnetic field with the wave field of the particle is

$$\mu_n \int (\psi^\dagger [\rho_3(\mathcal{H}\boldsymbol{\sigma}) - \rho_2(\mathcal{E}\boldsymbol{\sigma})]\psi)\, d\tau = \mu_n \int \{ -[\Psi^\dagger(\mathcal{H}\boldsymbol{\sigma})\Psi] + (\Phi^\dagger[\mathcal{H}\boldsymbol{\sigma})\Phi]\}\, d\tau + i\mu_n \int \{[\Phi^\dagger(\mathcal{E}\sigma)\Psi] - [\Psi^\dagger(\mathcal{E}\boldsymbol{\sigma})\Phi]\}\, d\tau, \quad (10)$$

it being understood that $\boldsymbol{\sigma}$ is a four-component Dirac $\boldsymbol{\sigma}$ when it occurs with ψ and that it is a two-component Pauli $\boldsymbol{\sigma}$ when it goes with Ψ or Φ. For low-particle momenta $\Phi \ll \Psi$ and only the first term in the first integral in the above formula for the interaction energy is appreciable. The interaction energy is then

$$-\mu_n \int [\Psi^\dagger(\mathcal{H}\sigma)\Psi]\, d\tau = -\mu_n \int [\mathbf{A} \cdot \operatorname{curl} (\Psi^\dagger \boldsymbol{\sigma} \Psi)]\, d\tau, \quad (10.1)$$

the transformation leading to the second form in Eq. (10.1) involving only a partial integration. Comparing this interaction energy with the standard form

$$-(1/c) \int (\mathbf{J} \cdot \mathbf{A})\, d\tau,$$

where $\mathbf{J}$ is the electric current density one sees that one may associate the current

$$\mathbf{J}^{(\mu)} = c\mu \operatorname{curl} (\Psi^\dagger \boldsymbol{\sigma} \Psi), \quad (10.2)$$

with the interaction energy of Eq. (10). On the other hand the particle current density for low momenta is

$$\mathbf{j}^{(\text{part})} = (\hbar/2Mi)[\Psi_\alpha^* \nabla \Psi_\alpha - \Psi_\alpha \nabla \Psi_\alpha^*] + (\hbar/2M) \operatorname{curl} (\Psi^\dagger \boldsymbol{\sigma} \Psi). \quad (10.3)$$

The first part of this expression is the same in form as the non-relativistic Schroedinger current summed over both spin orientations. The second is the spin current. Comparison of Eq. (10.2) with Eq. (10.3) shows that for low kinetic energies of the particle Eq. (1.1) gives an interaction energy which would be obtained if the electric current density were equal to a constant times the spin particle current density.

If the form of the interaction energy is known in the rest system of the particle then by a Lorentz transformation one knows it also in other systems and hence the form of the interaction energy in Eq. (1.1) may be considered as following from the supposition that in a system in which the particle momentum is small the electric and the spin current densities are proportional to each other with a proportionality constant independent of the externally applied electromagnetic field. Since the neutron has presumably a size in some sense the electric current density can hardly be describable exactly in the above manner. The size can hardly be important, however, for the relatively slow variations of the electric field which occur in terms of an atomic length scale in experiments on the electron-neutron interaction.

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Summary.—The explanation of the electron-neutron interaction advanced by Foldy is analyzed without the employment of the special canonical transformation of Foldy and Wouthuysen. The meaning of the Pauli magnetic moment term is illustrated by a consideration of special cases and of the equations of motion which show how the term corresponds to the visualization of a classical magnetic doublet oriented along the spin of the neutron. The interaction of the neutron moment with the divergence of the electric field is compared with a similar effect in a phenomenologic treatment of the electron's magnetic moment which has previously been calculated. The more closely relativistic treatment of the latter calculation enables one to express the answer in terms of Dirac functions rather than their Schroedinger approximations. Assumptions sufficient for the applicability of Foldy's explanation independently of details of the origin of the neutron's magnetic moment are discussed.

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[2] Breit, G., *Ibid.*, **72**, 984 (1947); **73**, 1410 (1948); **74**, 656 (1948). The last of the papers just quoted has the more complete treatment. It should be mentioned that a closely related effect appears in the paper by H. Gaus, *Zeitsch. f. Naturforschung*, **4a**, 721 (1949). Gaus reduces to a two-component form the mesonic generalization of Pauli's intrinsic moment equation which has been set up by Bethe in 1939. He obtains a term $-\hbar f\Delta\varphi^0/(2mc\kappa)$ which is the generalization to mesonic problems of the effect principally discussed by Foldy and in the present paper. The vector meson theory used by Gaus is apparently in poor agreement with the newer experimental evidence. The

term calculated by Gaus is nevertheless of interest because it implies the interaction of a particle with the density of other particles and can be made to have either sign by a suitable choice of sign of the interaction constant f. If present, this term would seriously affect considerations regarding the saturation of nuclear forces.

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FINDING THE SIGNS OF THE F's FROM THE SHIFTED PATTERSON PRODUCT

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Many recently proposed methods for the determination of a crystal structure fall into one of two classes: (*a*) The determination of the phases of the coefficients in the fourier series representing the structure and (*b*) the deciphering of its Patterson synthesis. The present communication combines the experience of two workers, one from each of these two fields; its purpose is to show that the method of Patterson deciphering known as "Mixed Projections" by multiplication (i.e., "shifted products") leads to a new and very powerful equality which relates the F's of a crystal to its F^2's. This equality is restricted at present to centrosymmetric structures in which all the atoms are alike and which lead to Patterson functions having neither overlapping maxima nor multiple peaks except those doubled by the center of symmetry. It is hoped that corresponding relations may be found for more general cases.

The basis of the new method to be presented here is the fact that the electron density distribution in atoms is nearly gaussian, and that the square of the Patterson function of a gaussian atom is also gaussian and has the same width at half height. This last can be shown as follows:

The electron density in an atom at the origin may be represented by a gaussian function such as

$$\rho(\mathbf{r}) = ae^{-br^2} \tag{1}$$

where $\rho(\mathbf{r})$ is the electron density at a vector distance $\mathbf{r}$ from the origin, $r = |\mathbf{r}|$, a is a constant related to the total number of electrons in the atom and b is another constant inversely proportional to the square of the atom's width at half height.

The Patterson function $P(\mathbf{u})$ of such an atom is

$$P(\mathbf{u}) = \int_{\text{over } V_r} \rho(\mathbf{r})\rho(\mathbf{r}+\mathbf{u})dV_r = a^2\sqrt{\left(\frac{\pi}{2b}\right)^3}\, e^{-1/2\, bu^2} \qquad (2)$$

where $\mathbf{u}$ is the position vector in Patterson space and $u = |\mathbf{u}|$.

The corresponding function for the "squared Patterson atom" is

$$P^2(\mathbf{u}) = a^4\left(\frac{\pi}{2b}\right)^3 e^{-bu^2}. \qquad (3)$$

A comparison of equations (1), (2) and (3) shows that the width of the Patterson atom at half height is $\sqrt{2}$ times as great as that of the corresponding atom, *whereas the width of the "squared" Patterson atom is the same as that of the atom.* These statements are in agreement with empirical results obtained from crystals containing carbon atoms.[1] Perhaps even more important is that equations (1) and (3) indicate that "squared" Patterson atoms have the same shapes as atoms, i.e., at $\mathbf{u} = \mathbf{r}$

$$P^2(\mathbf{u}) = K\rho(\mathbf{r}), \qquad \text{where } K = \left(\frac{\pi a}{2b}\right)^3. \qquad (4)$$

Equation (4) reveals incidentally that the maxima found in the product of two mutually translated Patterson syntheses are the same shape—and therefore as easily resolvable—as those in a fourier synthesis of the corresponding crystal structure, provided the mutual translation brings Patterson maxima into exact superposition.

If $\rho(\mathbf{r})$ is the electron density of an atom centered at the origin, then $\rho(\mathbf{r} - \mathbf{r}_j)$ is that of an atom centered at $\mathbf{r}_j$. A centrosymmetric structure $\rho_S(\mathbf{r})$ containing N like atoms may be represented by two sets of atoms shifted equal and opposite distances from the origin, thus:

$$\rho_S(\mathbf{r}) = \sum_{j=1}^{N/2} [\rho(\mathbf{r} - \mathbf{r}_j) + \rho(\mathbf{r} + \mathbf{r}_j)]. \qquad (5)$$

The corresponding Patterson function is

$$P_S(\mathbf{u}) = \sum_{j=1}^{N/2}\sum_{k=1}^{N/2} [P(\mathbf{u} + \mathbf{r}_j + \mathbf{r}_k) + 2P(\mathbf{u} + \mathbf{r}_j - \mathbf{r}_k) + P(\mathbf{u} - \mathbf{r}_j - \mathbf{r}_k)] \qquad (6)$$

where $P(\mathbf{u})$ is defined as in equation (2).

Suppose that a certain atom at $\mathbf{r}_l$ and its centrosymmetric mate at $-\mathbf{r}_l$ are selected, and that the Patterson function (6) is shifted, so that its origin is at $\mathbf{r}_l$ in one case and at $-\mathbf{r}_l$ in another. The product $M_l(\mathbf{u})$ of these two shifted Patterson functions is given by:

$$M_l(\mathbf{u}) = P_S(\mathbf{u} - \mathbf{r}_l)P_S(\mathbf{u} + \mathbf{r}_l)$$
$$= (N-4)[P^2(\mathbf{u} - \mathbf{r}_l) + P^2(\mathbf{u} + \mathbf{r}_l)] +$$

$$+ 4 \sum_{j=1}^{N/2} [P^2(\mathbf{u} - \mathbf{r}_j) + P^2(\mathbf{u} + \mathbf{r}_j)]$$

$$+ \text{small terms.} \tag{7}$$

The small terms are due to the products of maxima with the "gaussian fringes" of other maxima; they may safely be neglected. Equation (7) is therefore "exact" if the Patterson function produces no overlapping maxima other than those due to the center of symmetry. By the use of equation (4), equation (7) may be rewritten

$$M_l(\mathbf{u}) = 4K\rho_S(\mathbf{u}) + (N - 4)K[\rho(\mathbf{u} - \mathbf{r}_l) + \rho(\mathbf{u} + \mathbf{r}_l)]. \tag{8}$$

Each term in equation (8) can be expanded in fourier series, thus:

$$\rho(\mathbf{u}) = \frac{1}{V_0} \sum_{\mathbf{h}} f(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u} \tag{9}$$

$$\rho_S(\mathbf{u}) = \frac{1}{V_0} \sum_{\mathbf{h}} F(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u} \tag{9'}$$

$$M_l(\mathbf{u}) = \frac{1}{V_0^2} \sum_{\mathbf{h}} G_l(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u} \tag{9''}$$

where V_0 is the volume of the unit cell. Then, by equating corresponding coefficients one obtains:

$$\frac{G_l(\mathbf{h})}{V_0} = 4KF_S(\mathbf{h}) + 2(N - 4)Kf(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{r}_l. \tag{10}$$

The fourier series for $P(\mathbf{u})$ is given by

$$P(\mathbf{u}) = \frac{1}{V_0} \sum_{\mathbf{h}} F^2(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u}. \tag{11}$$

From this, the coefficients $G_l(\mathbf{h})$ can be derived, thus:

$$P(\mathbf{u} - \mathbf{r}_l) = \frac{1}{V_0} \sum_{\mathbf{h}} C_l(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u} + \frac{1}{V_0} \sum_{\mathbf{h}} S_l(\mathbf{h}) \sin 2\pi\mathbf{h}\cdot\mathbf{u} \tag{12}$$

and

$$P(\mathbf{u} + \mathbf{r}_l) = \frac{1}{V_0} \sum_{\mathbf{h}} C_l(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{u} - \frac{1}{V_0} \sum_{\mathbf{h}} S_l(\mathbf{h}) \sin 2\pi\mathbf{h}\cdot\mathbf{u} \tag{12'}$$

where

$$C_l(\mathbf{h}) = F^2(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{r}_l \tag{13}$$

and

$$S_l(\mathbf{h}) = F^2(\mathbf{h}) \sin 2\pi\mathbf{h}\cdot\mathbf{r}_l. \tag{13'}$$

Multiplying, we obtain:

$$M_l(\mathbf{u}) = P(\mathbf{u} - \mathbf{r}_l)P(\mathbf{u} + \mathbf{r}_l)$$

$$= \frac{1}{V_0^2}\sum_{\mathbf{h}}\sum_{\mathbf{h}'} C_l(\mathbf{h})C_l(\mathbf{h}') \cos 2\pi\mathbf{h}\cdot\mathbf{u} \cos 2\pi\mathbf{h}'\cdot\mathbf{u}$$

$$- \frac{1}{V_0^2}\sum_{\mathbf{h}}\sum_{\mathbf{h}'} S_l(\mathbf{h})S_l(\mathbf{h}') \sin 2\pi\mathbf{h}\cdot\mathbf{u} \sin 2\pi\mathbf{h}'\cdot\mathbf{u}. \qquad (14)$$

Manipulation of formula (14) leads to the result:

$$M_l(\mathbf{u}) = \frac{1}{V_0^2}\sum_{\mathbf{h}}\left\{\sum_{\mathbf{h}'} F^2(\mathbf{h}')F^2(\mathbf{h}' - \mathbf{h}) \cos 2\pi(2\mathbf{h}' - \mathbf{h})\cdot\mathbf{r}_l\right\} \cos 2\pi\mathbf{h}\cdot\mathbf{u}. \qquad (15)$$

This means that the coefficients $G_l(\mathbf{h})$ in formula (9″) are given by:

$$G_l(\mathbf{h}) = \sum_{\mathbf{h}'} F^2(\mathbf{h}')F^2(\mathbf{h}' - \mathbf{h}) \cos 2\pi(2\mathbf{h}' - \mathbf{h})\cdot\mathbf{r}_l. \qquad (16)$$

The Use of the Foregoing Relations.—Suppose the x-ray diffraction data from a centrosymmetric crystal consisting of like (or almost like) atoms to have been converted into a table of absolute values of $F^2(\mathbf{h})$. From these values the Patterson function is calculated, using formula (11). This function exhibits maxima, some of which are at vector distances from the origin $2\mathbf{r}_l$; these maxima are among the smaller ones. One of these is selected and the values of $G_l(\mathbf{h})$ are obtained by the use of formula (16). It is now possible to use formula (10) in the form

$$F(\mathbf{h}) = \frac{1}{4K}\frac{G_l(\mathbf{h})}{V_0} - \left(\frac{N}{2} - 2\right) f(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{r}_l \qquad (17)$$

from which the sign of each $F(\mathbf{h})$ can be determined, since $K = \left(\frac{a\pi}{2b}\right)^3$, and $f(\mathbf{h})$ is given by

$$f(\mathbf{h}) = \int_{\text{over } V\mathbf{r}} \rho(\mathbf{r})e^{2\pi i\mathbf{h}\cdot\mathbf{r}}\, dV_{\mathbf{r}} = a\sqrt{\left(\frac{\pi}{b}\right)^3}\, e^{-(\pi^2\mathbf{h}^2/b)}. \qquad (18)$$

Using (18), a and b can be deduced from a table of the (average) atomic scattering factor, such as is found in many reference books, or better by the use of A. J. C. Wilson's method of plotting the averages of $F^2(\mathbf{h})$ against $|\mathbf{h}|$.[2] With the sign of each $F(\mathbf{h})$ known, the fourier series for the electron density in the structure can be evaluated:

$$\rho_S(\mathbf{r}) = \frac{1}{V_0}\sum_{\mathbf{h}} F(\mathbf{h}) \cos 2\pi\mathbf{h}\cdot\mathbf{r} \qquad (9')$$

This completes the determination of the crystal structure.

[1] McLachlan, D., unpublished.

[2] Wilson, A. J. C., *Nature* (London), **150,** 152 (1942).

THE OPTICAL THEORY OF WILLARD GIBBS

By Lynde P. Wheeler

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Sixty-nine years ago this month at the fall meeting of this Academy in New York Willard Gibbs read a paper entitled "On the General Equations of Optics as Derived from the Electromagnetic Theory of Light." This paper which contains the greater part of the ideas characteristic of his viewpoint was published the following year in the February number of the *American Journal of Science* and, together with the two previous papers of 1882 and the two critical papers of 1888–1889 in the same journal, it constitutes the whole of his published developments in the field of optics.

In addition to this published material there exists in the Sterling Memorial Library of Yale University a considerable volume of manuscript notes covering the subject as he was accustomed to present it in his graduate courses. There is little however in these manuscript notes that is not implicit in the five papers named above, although the essential ideas are developed in a much extended form, particularly in the discussion of the dispersion of colors, double refraction in various crystalline media, and the phenomena of rotary polarization both natural and magnetic.

The point of view adopted in all of this published and unpublished material is so original and differs so much from the conventional Maxwellian development of the electromagnetic theory of light and yet is so entirely compatible with the known experimental facts, that it seems to me to merit a more critical examination than it has yet received. These ideas of Gibbs in optical theory are the only ones he ever put forth which have not become dominant in science. His points of view in thermodynamic equilibrium, in physical chemistry, in vector analysis and multiple algebra, and in statistical mechanics have certainly proved to be controlling ones in the further development of those branches of science, and hence it would seem pertinent to inquire into the reasons why his ideas in optics have not exerted a comparable influence.

In the minds of his contemporaries it would seem that the chief significance of Gibbs' work in optics lay in the support it furnished the electromagnetic theory of light against the classical elastic solid theories and, as the spectacular experiments of Hertz supplied a more direct and easily grasped substantiation of that theory, Gibbs' more involved theoretical argument did not receive the critical examination it deserves. Since that time, as the advent of the electron theory supplied additional postulates which went far to remove Gibbs' objections to the original Maxwellian point of view, the motive for a closer study of the ideas and methods of

these early papers was much weakened and they have continued to the present time to remain inadequately explored.

But now, when the complexities of our picture of the molecular and atomic structure of matter are leading to an increasingly complex formulation of optical theory from the Maxwellian point of view, it would appear not unreasonable—because of its simple postulates, its rigorous dynamical approach, and its qualitative accounting for the observed facts of optics—to undertake a too long delayed reconsideration of the Gibbsian method of attack. It is not beyond the bounds of possibility that, when interpreted in terms of modern ideas of atomic and molecular structure, it may yield a better understanding of some of the phenomena concerned.

In the development of his theory Gibbs was motivated by the desires, first, to avoid speculative and special hypotheses as to the structure of matter, and, second, to seek the simplest possible dynamical expression for the wave motions responsible for optical phenomena. His program of research here as always consisted in an exhaustive examination of all of the possibilities inherent in the simplest and most certain of basic physical and mathematical assumptions before invoking the aid of more speculative approaches. Thus here he predicates for the structure of matter only that it consists of identical cells small with respect to the wave length of the radiation which can traverse it but still large enough to contain many of the molecular elements of which it may be composed. Within each cell he supposes the *space* average of the motions of the constituent charged particles to be expressible by the ordinary equations of wave motion, while the motion of the individual particles (similarly expressed) might depart widely from that average. Thus he pictures the displacement of electricity within a cell as divided into two portions—that due to the average or *regular* motion, and that due to the actual molecular or *irregular* motion—each by the principle of superposition having the same period as that of the incident radiation, and the average of the irregular part vanishing when taken over the volume of a cell.

This simple model is as valid today as when originally proposed, requiring only a formulation of the irregular part of the motion in terms of the postulates of the electron theory to bring it into accord with modern viewpoints on the structure of matter.

Similarly for the relation between the electrical forces and the motions they must maintain within the cells, Gibbs supposes it to have the simplest possible dynamical form, namely that the force and the displacement are, for the *regular* part of the motion, linear vector functions each of the other; while for the *irregular* part the force is supposed additionally to be a similar function of the *space* rate of change of the displacement. The coefficients in terms of which these functions are expressed will depend on the nature of the medium and the frequency of the oscillating electric force. For

isotropic media not subject to magnetic influence, the coefficients may be taken to be numerical factors.

It should be noted that this vector relationship is one between the electric force propagated through the medium and the *total* resulting motion of electricity within a cell, whether that is elastic or convective or a combination of the two. Thus Gibbs' "displacement" is *not* in general the same as Maxwell's, which is a purely dielectric or elastic one. For media in which no convective motion of electricity can take place (i.e., in pure dielectrics) the Gibbsian and the Maxwellian "displacements" become identical, and hence the optical phenomena of transparent bodies will receive a like explanation from either point of view. But in media which support both kinds of motion (i.e., in absorbing media) the optical phenomena will receive a different interpretation according to which definition of "displacement" is adopted. Thus it is only in the phenomena of such media that we can look for evidence which will discriminate between the two hypotheses.

Consider first the differences in the formulation of the general equations which result from the alternative definitions of the displacement. Some of these are shown in table 1 below.

TABLE 1

	MAXWELL	GIBBS
Field Force—Displacement Relation	$E = D/\epsilon$	$E = \Phi U + \Psi \dot{U}$
Total Current Density	$J = \dot{D} + \sigma E = \epsilon \dot{E} + \sigma E = \dot{E}(\epsilon + i\sigma/\omega)$	$J = \dot{U} = \dot{E}/(\Phi - i\omega\Psi) = \dfrac{\dot{E}\,\Phi + i\omega\Psi}{\Phi^2 + \omega^2\Phi^2}$
Index of Refraction (Absorbing Media)	$n^2 = \epsilon_r + i\sigma/\epsilon_0\omega$	$n^2 = \dfrac{1}{\epsilon_0} \cdot \dfrac{\Phi + i\omega\Psi}{\Phi^2 + \omega^2\Psi^2}$

In this table I have distinguished between the two displacements by using the symbol D for that of Maxwell and U for that of Gibbs. E, D, U and J are supposed to be complex vectors (bivectors) whose real parts represent the actual physical magnitude concerned. That is they are of the type $V = V_r + iV_r/\omega = V_0 \exp \omega(s \cdot \rho - t)$, where $i = \sqrt{-1}$, ω is the angular frequency, ρ a position vector, and $s = 1/c$ is the wave *slowness*. Φ and Ψ are the Gibbsian linear vector functions which in the only case to be considered here may be taken to be constants playing rôles analogous to the dielectric constant ϵ and the conductivity σ of the Maxwellian formulation. ϵ_0 is the dielectric constant of free space, which in the units used (those of the "rationalized" m.k.s. system) has the value $\epsilon_0 = 10^7/4\pi c_0^2$, with $c_0 = 3(10)^8$ m/sec, approximately. ϵ_r is the *relative* dielectric constant, i.e., $\epsilon = \epsilon_r\epsilon_0$.

Although the Gibbsian expressions are seen to be more complicated

mathematically than the Maxwellian, the physical significance of Φ and Ψ as functions of the frequency (as well as of the structure of the medium) immediately suggests the necessity of determining the law of their frequency dependence; whereas the simpler Maxwellian expressions contain no such hint with respect to ϵ and σ. In fact it was not until long after Maxwell's time that the advent of the electron theory brought a realization of such a necessity, and expressions for ϵ and σ as functions of frequency were found. That Gibbs realized this necessity in 1882 is a striking instance of the comprehensiveness of his thinking.

Next, let us consider briefly some of the consequences of the two formulations in particular cases. These are shown in table 2.

TABLE 2

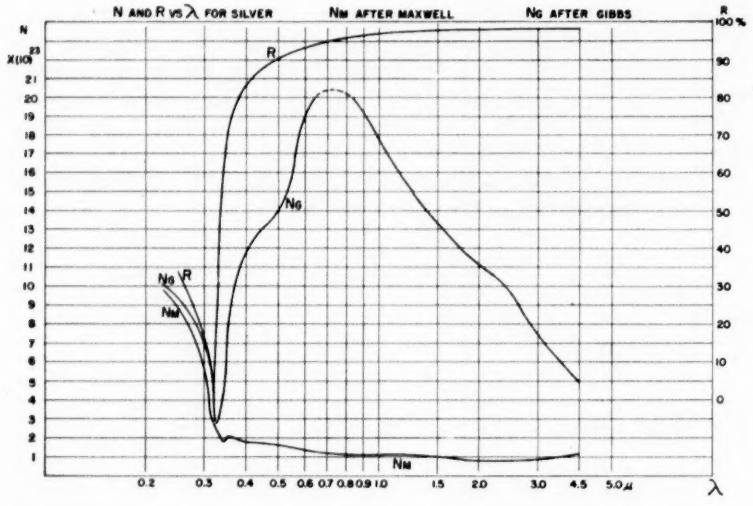
	MAXWELL	GIBBS
In a pure Dielectric Medium	$\epsilon = J_a/E = D/E$	$1/\Phi = J_a/E = U/E$
In a pure Conductive Medium	$\sigma = J_c/E$	$1/\Psi = \omega J_c/iE = J_c/E$
In an Absorbing Medium	$\epsilon = \epsilon_0(\nu^2 - \kappa^2)$	$1/\Phi = \epsilon_0(\nu^2 + \kappa^2)^2/(\nu^2 \cdot \kappa^2)$
	$\sigma = \epsilon_0\omega 2\nu\kappa$	$1/\Psi = \epsilon_0\omega(\nu^2 + \kappa^2)^2/2\nu\kappa$
In Metallic Reflection	$N \backsim \sqrt{\frac{2\nu\kappa}{\lambda^3}}$	$N \backsim \sqrt{\frac{1}{\lambda^3} \cdot \frac{(\nu^2 + \kappa^2)^2}{2\nu\kappa}}$

In this table J_a is the dielectric and J_c the conduction current density; ν is the index of refraction, κ the coefficient of extinction, N the number of conduction electrons per unit volume and λ the wave length of the incident radiation. The first line of this table shows formally what has been indicated previously, namely that the two formulations become identical for a pure dielectric. The second line shows that the same is true for a pure conductor. In the third line are shown the relations between the optical and electrical parameters of an absorbing medium; those following from the Maxwellian formulation being of course well known, while those from the Gibbsian are here published for the first time, although they appear in Gibbs' manuscript notes in the Yale Library.

The expressions of the fourth line of the table follow from those of the third on introducing for σ that value expressed as a function of frequency which is yielded by the electron theory of metallic conduction. It is to these divergent expressions for the conduction electron density that I wish to call particular attention.

Using the experimentally determined values of the optical parameters for the case of silver (which have been measured over a wide spectral range), I have computed the values of N demanded by both of the formulations with the result as shown in the following figure, in which is also included the experimental curve for the normal incidence reflection coefficient taken from the measurements of Hagen and Rubens and of Coblenz. In this figure the curve marked R is for the reflection coefficients, and those marked

N_M and N_G for the conduction electron densities (the numbers per cm.^{3}) yielded by the Maxwellian and the Gibbsian formulations respectively.



From the curve for N_M we would have to conclude that the conduction electron density effective in the phenomena of metallic reflection for silver decreases continuously with decreasing frequency, at first rapidly through a resonance region in the ultraviolet (at which point the metal is quite transparent), and then more slowly through the visible and infrared portions of the spectrum. This latter part of the curve which shows a smaller density of electrons than at the point of maximum transparency would seem to be difficult to reconcile with the great opacity and reflecting power of the metal in the longer wave length regions.

On the other hand, the curve N_G representing the Gibbsian viewpoint shows a minimum of the conduction electron density at the precise point in the spectrum where experiment shows that a minimum of reflecting power occurs. This is followed by a maximum in the near infrared with a subsequent decrease in the longer infrared region. This curve would seem to be largely free from the objection cited above to the Maxwellian curve in that the variation in the computed conduction electron densities are much more closely correlated with the experimentally observed reflection coefficients. Neither curve can pretend to tell the whole story owing to the neglect of certain factors in the computations, but they do represent the results to be expected on each of the two formulations to the *same* degree of approximation.

Hence it appears that we have here valid evidence that the Gibbsian definition of "displacement" leads in the case of the phenomena of metallic reflection to results more compatible with the experimental facts than does the Maxwellian. This seems to me to justify a comprehensive reconsideration of all of the phenomena of absorbing media in the light of the Gibbsian definition, and suggests that even at this late date we still have something to learn from the paper first presented to this body in 1882.

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*OXIDATIVE AND PHOSPHORYLATIVE ACTIVITY OF PLANT MITOCHONDRIA**

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The experiments reported in this paper concern the mechanisms involved in plant respiration, particularly those involved in the oxidation of pyruvate. The interest of such a study lies in the fact that although pyruvate is well established as an intermediate in the respiratory oxidation of hexose by plant tissues,[1] it has not heretofore been possible to bring about the further oxidation of pyruvate *in vitro* by any enzyme system of plant origin. Studies of the malonate inhibition of plant respiration *in vivo* have indicated that, in the plant as in animal tissues, pyruvate oxidation is accomplished through the reactions of the Krebs cycle with the participation of a malonate-inhibitable succinoxidase system.[2] The earlier work of Laties[3] with cauliflower, of Millerd[4] with potato and of Bhagvat and Hill[5] with various species has established that the succinoxidase activity of plant tissue is associated with the particulate components of the cytoplasm. It has been found in the present work that a variety of further respiratory enzymes are associated with the same particles. From seedlings of the mung bean, *Phaseolus aureus*, particles have been isolated which are capable of carrying out all of the reactions of the Krebs cycle including the complete oxidation of pyruvate to CO_2 and water.

During the separation of the particulate matter attention must be paid to osmotic concentration, pH and phosphate concentration of the dispersion medium and the temperature at which the operations are conducted must be kept close to 0°C. The cytoplasmic particles with which we are concerned are sedimented in 15 minutes by a gravitational field of 10,000 g. after they have been prepared under these conditions. They

are approximately 0.5 to 2 μ in diameter and thus possess the dimensions characteristic of plant mitochondria. That the active particles are indeed mitochondria is established by the fact that they stain brilliantly with Janus Green B (dye concentration 1:50,000), a property which is unique to intact mitochondria.[6]

Finally, the oxidative metabolism of these plant mitochondria may be coupled to the generation of energy rich phosphate in the form of adenosine triphosphate, ATP, providing a channel through which the energy liberated in respiratory oxidations may be made available to energy consuming reactions of the cell.

Materials and Methods.—Etiolated seedlings of *Phaseolus aureus* were grown at 26°C. under a low intensity red light. The seeds were first sterilized by immersion for 10 minutes in 0.5% NaOCl, soaked in distilled water for 60 minutes, and then planted in vermiculite which had been previously saturated with water. The seedlings were harvested 90 hr. later at which stage they were approximately 8 cm. in length.

Thirty gm. of tissue representing the complete aerial portion of the plant were ground for approximately 2 minutes in a mortar with 10 gm. sand and 40 ml. of 0.1 *M* phosphate (KH_2PO_4 and Na_2HPO_4), pH 7.1, containing 0.4 *M* sucrose (solution 1). The brei was strained through muslin and clarified by low-speed centrifugation (500 g. for 5 minutes). The residual suspension was then recentrifuged (10,000 g. for 15 minutes) and the supernatant removed by suction. The residue was resuspended in 20 ml. of solution I and recentrifuged (10,000 g. for 15 minutes), the supernatant again removed by suction and the residue resuspended in 3.5 ml. of solution I. One-half ml. of this preparation, containing from 0.55 to 0.7 mg. N, was used as the enzyme in the reaction mixture which in all cases had a total volume of 1.5 ml. All steps in the preparation were carried out at 2°C. or lower while measurements of the metabolic activities of the particles were carried out at 30°C. Metabolic measurements were continued for a period of one hour unless otherwise stated. The methods used in this preparation are based essentially on the recommendations of Laties.[3]

Oxidative Ability of Plant Mitochondria.—The oxidations carried out by mitochondrial preparations result in the consumption of oxygen and may be followed manometrically. The data of table 1 show that principal acids of the Krebs cycle are oxidized by the system. In this experiment the rates of oxidation of the various substrates, determined under conditions appropriate for maximal rate of pyruvate oxidation, may be compared. It is evident however that citrate, α-ketoglutarate, succinate and pyruvate are oxidized more rapidly than are malate and fumarate.

For the oxidation of pyruvate, the simultaneous oxidation of a small amount of one of the acids of the Krebs cycle, as for example malate, is

essential as is shown in table 2. The respiratory quotient for the oxidation of pyruvate under these circumstances is 1.3, corresponding to the expected value for the complete oxidation to CO_2 and water.

Other acids of the Krebs cycle may be substituted for malate in its role of promoting the oxidation of pyruvate. The data of table 3 show that these acids are approximately equally effective in this function.

TABLE 1

OXIDATION OF ACIDS OF THE KREBS CYCLE BY CYTOPLASMIC PARTICLES OF MUNG BEAN

REACTION MIXTURE[a]	SUBSTRATE CONCENTRATION	O_2 CONSUMPTION, CU. MM./HR./MG. N
Enzyme	...	0
Enzyme + citrate	2×10^{-2} *M*	208
Enzyme + α-keto-glutarate	2×10^{-2} *M*	220
Enzyme + succinate	2×10^{-2} *M*	202
Enzyme + fumarate	2×10^{-2} *M*	23
Enzyme + l-malate	2×10^{-2} *M*	54
Enzyme + l-malate	1.7×10^{-3} *M*	7
Enzyme + l-malate[b] + pyruvate	2×10^{-2} *M*	105

[a] Also contains sucrose 0.3 *M*, phosphate buffer 0.05 *M*, ATP 5×10^{-4} *M* and $MgSO_4$ 10^{-3} *M*.

[b] Malate concentration, 1.7×10^{-3} *M*.

TABLE 2

CATALYTIC EFFECT OF MALATE ON PYRUVATE OXIDATION

REACTION MIXTURE[a]	GAS EXCHANGE (CU. MM./HR./MG. N): O_2	CO_2
Enzyme	15	5
Enzyme + malate[b]	31	24
Enzyme + pyruvate[c]	16	36
Enzyme + malate + pyruvate	164	226

R. Q. for pyruvate oxidation = 1.3.

[a] Also contains sucrose 0.3 *M*, phosphate buffer 0.05 *M*, ATP 5×10^{-4} *M* and $MgSO_4$ 10^{-3} *M*.

[b] l-Malate 1.7×10^{-2} *M* throughout.

[c] Pyruvate 2×10^{-2} *M* throughout.

Cofactor Requirements of Plant Mitochondria.—The cytoplasmic particles of mung bean, when appropriately prepared, are strikingly independent and require no additions of coenzymes or other cofactors other than catalytic amounts of a Krebs cycle acid in order to oxidize pyruvate. Thus the particles exhibit no requirement of or response to added cytochrome c, a property in which they differ strikingly from particulate

preparations of plant succinoxidase such as have been recently described.[5, 7] This lack of requirement for exogeneous cytochrome c appears to be associated with the integrity of the present material and is apparently related to the method of preparation since by a slight variation in the method, namely by omitting or lowering the concentration of phosphate in the grinding medium, it is possible to obtain particles which do respond

TABLE 3

CATALYTIC EFFECT OF KREBS CYCLE ACIDS ON PYRUVATE OXIDATION[a]

KREBS CYCLE ACID ADDED	CONCENTRATION, M	O_2 CONSUMPTION IN PRESENCE OF ACID ALONE	CU. MM./HR./MG. N IN PRESENCE OF 2×10^{-2} M PYRUVATE
None	....	0	0
Fumarate	1.7×10^{-3}	3	94
Malate	1.7×10^{-3}	18	93
Succinate	1.7×10^{-3}	18	112
α-Ketoglutarate	1.7×10^{-3}	20	100
Citrate	1.3×10^{-3}	21	45

[a] Reaction mixture contains enzyme, sucrose 0.3 M, phosphate buffer 0.05 M, ATP 5×10^{-4} M and $MgSO_4$ 10^{-3} M.

TABLE 4

RELATION OF METHOD OF PREPARATION TO CYTOCHROME C REQUIREMENT OF CYTOPLASMIC PARTICLES

Standard Method

REACTION MIXTURE[a]	O_2 CONSUMPTION, CU. MM./HR./MG. N
Enzyme	0
Enzyme + succinate (2×10^{-2} M)	202
Enzyme + succinate (2×10^{-2} M) + cytochrome c (10^{-5} M)	204

Modified Method[b]

REACTION MIXTURE[c]	O_2 CONSUMPTION, CU. MM./HR./MG. N
Enzyme	0
Enzyme + succinate (2×10^{-2} M)	7
Enzyme + succinate (2×10^{-2} M) + cytochrome c (10^{-5} M)	45

[a] Also contains sucrose 0.3 M, and phosphate buffer 0.05 M.
[b] Phosphate omitted from initial grinding mixture.
[c] Also contains sucrose 0.2 M, and phosphate buffer 0.05 M.

to added cytochrome c as is shown in table 4. The oxidations carried out by the system are therefore apparently mediated by the cytochrome system.

Although the only supplement to the reaction mixture required for the oxidation of pyruvate by mung bean particles is a catalytic amount of one of the acids of the Krebs cycle, still the rate of oxidation is increased

by the addition of further substances to the reaction mixture. The materials which exhibit such a quantitative influence on rate of substrate oxidation are ATP and Mg ions. The data of table 5 show that these two materials are effective individually but that together they may increase rate of pyruvate oxidation threefold or more. This effect is not unique to ATP but is shared equally by ADP, adenosinediphosphate, and AMP, adenosine-5-phosphate. Adenosine-3-phosphate, yeast adenylic acid, is totally ineffective. These relations are summarized in table 6.

TABLE 5

EFFECT OF CATALYTIC AMOUNTS OF ATP AND OF MG IONS ON THE RATE OF PYRUVATE OXIDATION BY CYTOPLASMIC PARTICLES

REACTION MIXTURE[a]	O_2 CONSUMPTION, CU. MM./HR./MG. N
Enzyme + pyruvate + malate	60
Enzyme + pyruvate + malate + ATP	97
Enzyme + pyruvate + malate + Mg ions	135
Enzyme + pyruvate + malate + ATP + Mg ions	189

[a] Also contains sucrose 0.3 *M*, and phosphate buffer 0.05 *M*. 1-Malate, 1.7×10^{-3} *M* and pyruvate 2×10^{-2} *M*, throughout.

TABLE 6

EQUIVALENCE OF ATP, ADP AND AMP IN INCREASING THE RATE OF PYRUVATE OXIDATION BY CYTOPLASMIC PARTICLES

SUPPLEMENT TO REACTION MIXTURE[a]	O_2 CONSUMPTION, CU. MM./HR./MG. N
Enzyme	58
Enzyme + ATP (10^{-3} *M*)	111
Enzyme + ADP (10^{-3} *M*)	107
Enzyme + AMP (10^{-3} *M*)	102
Enzyme + adenosine-3-phosphoric acid (10^{-3} *M*)	58

[a] Contains pyruvate 2×10^{-2} *M* as the substrate. Also contains 1-malate 1.7×10^{-3} *M*, sucrose 0.3 *M*, phosphate buffer 0.05 *M* and $MgSO_4$ 10^{-3} *M*.

The standard method for the preparation of cytoplasmic particles active in the oxidation of the Krebs cycle acids includes the use of phosphate in the medium in which the initial grinding is carried out. The presence of phosphate in the grinding medium appears to be essential if active particles are to be obtained from mung bean. In addition, however, the presence of phosphate in the reaction mixture is essential to maximum oxidative activity as is shown in figure 1. In this experiment, the particles were prepared in phosphate medium according to the standard procedure, washed free of phosphate by centrifugation in a solution of sucrose and KCl, and the rate of oxidation of α-ketoglutarate determined as a function of phosphate concentration in the reaction

mixture. This and other data show a well-marked optimum phosphate concentration in the neighborhood of 0.01 *M*. None of the preparations obtained were entirely free of inorganic phosphate and therefore an absolute requirement of this system for phosphate has not been established.

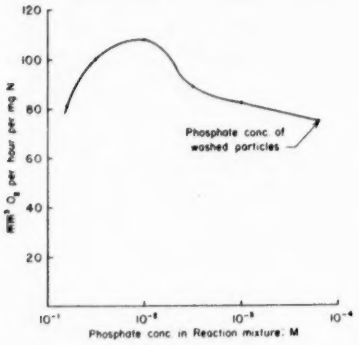

FIGURE 1

Rate of oxidation of α-ketoglutarate by mung bean particles as a function of phosphate concentration in the reaction mixture. Reaction mixture also contains sucrose 0.3 *M*, KCl 0.05 *M*, $MgSO_4$ 10^{-3} *M*, AMP 10^{-3} *M* and α-ketoglutarate 2×10^{-2} *M*.

Phosphorylative Ability of Plant Mitochondria.—The facts which have been presented suggest, of course, that mitochondrial oxidations may be linked in the plant as they are in the animal to the production of ATP. That this might be the case is intimated not only by the requirement of the particles for inorganic phosphate during the course of substrate oxidation but also by the requirement for ATP or related materials. Detection of ATP synthesis is complicated by the fact that the particles contain an ATPase highly active in the removal of labile phosphate from ATP. Fortunately this enzyme can be inhibited 90% or more by fluoride ions at a concentration of 0.01 *M*, a concentration which leaves substrate oxidation unaffected. It has now been possible to show unequivocally that substrate oxidation results in the incorporation of inorganic P into ATP. For this experiment plant mitochondria were allowed to oxidize α-ketoglutarate in the presence of ATP and P^{32} labeled inorganic phosphate. A portion of the ATP added was degraded to ADP by a mechanism to be discussed below making possible the resynthesis of ATP at the expense of inorganic phosphate of the reaction mixture. After a period of reaction, the ATP was reisolated by two dimensional paper chromatography.[8] The ATP now contained P^{32} as is shown in table 7. Appearance of inorganic phosphate in energy-rich form was almost wholly dependent on the simultaneous oxidation of substrate as is shown in table 7. The production of energy-rich phosphate bonds as a result of oxidations of the Krebs cycle not only occurs in the higher plant but also follows the general pattern already elucidated for animal tissues.

Plant mitochondria not only possess the ability to generate ATP at the expense of energy derived from substrate oxidation but they also appear capable of utilizing ATP for at least one synthetic reaction. The enzyme, hexokinase, which catalyzes the reaction between ATP and hexose to form hexose-6-phosphate is associated with the cytoplasmic particles

of mung bean, as prepared by the present technique. Thus in the experiment, table 7, P^{32} labeled hexose phosphate as well as ATP appeared in the reaction mixture during the course of substrate oxidation. Hexose, derived from the sucrose of the suspending medium by the action of particle-bound invertase, served as the substrate for mitochondrial hexokinase. The participation of phosphorylase in this reaction is excluded since labeled hexose phosphate appeared only as a result of substrate oxidation.

Discussion and Summary.—The respiratory oxidation of pyruvate to CO_2 and water has been achieved with an enzyme system prepared from a higher plant, the mung bean. The enzymes involved are all associated with cytoplasmic particles which appear to be identical with mitochondria. It is of special interest in this connection that the oxidase system associated with these particles and hence responsible for the respiratory oxidation of pyruvate in this plant preparation is apparently the cytochrome system. The presence of the cytochrome system in plants is well known although it is equally true that other oxidase systems are present in many plant tis-

TABLE 7

INCORPORATION OF INORGANIC P^{32} INTO ATP AND HEXOSE PHOSPHATE DURING MITOCHONDRIAL OXIDATION[a]

SUBSTRATE	O_2 CONSUMPTION, CU. MM. O_2/30 MIN.	% OF INORGANIC P^{32} INCORPORATED INTO: ATP	PHOSPHOHEXOSES	RELATIVE SPECIFIC ACTIVITY OF ATP[b] %
None	0	0.04	0.26	0.85[c]
α-Ketoglutarate (2×10^{-2} M)	86	2.24	1.79	47.5

[a] Reaction mixture contained enzyme, sucrose 0.3 M, phosphate buffer 0.05 M, $MgSO_4$ 10^{-3} M, ATP 10^{-3} and NaF 10^{-2} M.

[b] In labile P of ATP only.

[c] Relative specific activity of inorganic P in reaction mixture = 100%.

sues. It has been inferred from inhibition experiments *in vivo* that the cytochrome system mediates a portion or all of the respiration of certain plant tissues. The findings presented above however now directly implicate the cytochrome system in one of the major respiratory processes, the complete oxidation of pyruvate. The fact that mitochondrial oxidation of pyruvate requires the simultaneous oxidation of an acid of the Krebs cycle, as well as the ability of the particles to oxidize these materials, provides further support for the view, based on *in vivo* experiments that respiratory oxidation of pyruvate in the plant as in the animal may proceed by the successive steps of the Krebs' cycle.[2]

Mitochondrial oxidation by the mung bean preparation has been linked to the formation of energy-rich phosphate bonds in the form of ATP. This fact, together with the close similarity or identity of higher plant ATP to that of animals and microorganisms,[9] indicates that the mechanics

of energetic coupling in the higher plant are not dissimilar from those of animal tissues.

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** Predoctoral Fellow, French Foundation. Recipient of Fulbright travel grant, administered by the Institute of International Education.

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HYBRIDIZATION AND EMBRYONIC TEMPERATURE ADAPTATION STUDIES OF RANA TEMPORARIA AND RANA SYLVATICA

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Rana temporaria and similar medium-sized, black-masked frogs are found throughout nearly the entire Holarctic region. In Europe and Asia the common species are *Rana temporaria*, *Rana arvalis*, *Rana agilis*, *Rana japonica* and *Rana choachiaoensis*. In North America the representatives are *Rana sylvatica* and perhaps several western species.

The close resemblance of *Rana sylvatica* and *Rana temporaria* has been noted by many authors. This resemblance could be an indication of genetic similarity or an independent development of like color patterns.

In an effort to differentiate between these alternative hypotheses, comparison of the two species on the basis of characters other than pigmentation is desirable. Previous work has shown that embryonic temperature tolerance and rate of development are highly species specific in the amphibia. Consequently it is of some interest to compare the embryonic temperature tolerance and rate of development of *Rana temporaria* with that of *Rana sylvatica*.

Another indication of relationship can be obtained from hybridization studies. Data of this sort, however, must be interpreted with caution. As far as frogs are concerned, successful hybridization occurs only between closely related species yet not all closely related species form hybrids.

The *Rana temporaria* used in these experiments were from two sources. Some were collected in the Vosges district of eastern France and others near Louvain, Belgium.[1] In both instances they were shipped to New York, via air express, for experimentation. From all appearances the animals were in top physiological condition when used. Embryos from three pairs of French and two pairs of Belgium *temporaria* were studied. The methods of experimentation were the same as those employed in other work of this type.[2]

Temperature Tolerance.—Development of the French embryos was ob-

TABLE 1

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *Rana temporaria*

The figures in the body of the table represent the earliest and latest hour at which 50% or more of a batch of embryos were in a given morphological stage. First cleavage (stage 3) is taken as 0 time.

STAGE	9.7°	12.7°	15.5°	19.5°	24.6°
3	0	0	0	0	0
7	17.5	...	...	4.0	...
8	23.0–30.1	17.4	17.2	...	9.8
9	36.3–46.0	24.0–30.7	...	...	...
10	51.5–66.2	29.9–30.7	24.0–30.8	17.3–20.0	17.0
11	71.5–78.5	42.1–46.3	...	23.9–24.0	...
12	77.1–95.0	51.5–56.0	36.5–42.0	25.9–30.5	20.0–24.0
13	95.0–106.5	...	46.0	30.5	22.8
14	116.0–143.0	66.3–78.3	51.6–56.0	36.1–41.8	25.7–30.5
16	136.7–165.8	88.5–95.0	66.3–71.5	44.3–46.0	30.0
17	168.8–174.3	106.3	77.3–78.3	51.5	36.5
18	211.0–261.3	115.3–153.7	88.6–106.3	56.0	41.5–51.0
19	263.5–285.7	...	106.3–115.2	66.0–73.8	53.0–55.9
20	283.0	160.0	117.3	74.0	58.5

served at 4.1°, 9.7°, 12.7°, 15.5°, 19.5°, 24.6°, 27.2° and 29.1° and of the Belgium embryos at 4.1°, 9.6°, 12.6°, 15.4°, 19.4°, 24.4°, 27.4° and 29.1°.[3] Although in some cases the temperatures at which the French and Belgium embryos kept were slightly different the results are combined. The lowest temperature, 4.1°, did not permit entirely normal development. In the case of all five groups of embryos cleavage and gastrulation were superficially normal. The neural folds were lower and the width of the anterior end of the neural plate less than is normal. Some embryos cytolized in stage 18 and 19.[4] The most conspicuous defects were of the gills. They were reduced in size, frequently the tips were swollen, and in 50% or more (depending on the group) gill circulation never commenced. This temperature of 4.1° was obviously very close to the lower limiting temperature for

the species, which is tentatively set at 5°. No deleterious effects of temperature were noted at 9.7°, 12.7°, 15.5° or 19.5°. At 24.6° high-temperature injuries occurred. In two groups of the French embryos less than half developed normally but in the third French group and both Belgium groups more than half exhibited normal development. Except for one Belgium group that gave 1% development at 27.2°, no embryos developed normally at either 27.2° or 29.1°. On the basis of these data the upper limiting temperature for *temporaria* is set at 25°. The temperature range for 50% or more normal development in *temporaria* is, therefore, 5–25°. The range for the American *Rana sylvatica* is 2.5–24°.[5] The temperature range for *temporaria* resembles that of *sylvatica* more closely than it does any other American *Rana*.

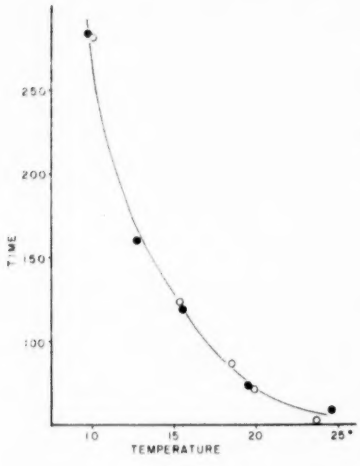

FIGURE 1

A comparsion of the speed of development in *Rana temporaria* (dots) *Rana sylvatica* (circles). Time signifies the hours required to develop from first cleavage (stage 3) to gill circulation (stage 20).

Rate of Development.—Table 1 shows the time required to reach various stages of development at five temperatures. No significant differences could be detected between any of the five groups of embryos studied. As a result all observations are combined in the table. At 4.1° there was a suggestion that the French embryos developed somewhat faster than those from Belgium. Since development at this temperature cannot be considered entirely normal this difference in rate may be disregarded.

In figure 1 a comparison is made of rate of development of *Rana temporaria* and *Rana sylvatica*. Data for the former are taken from table 1 and for the latter the source is a previous paper.[6] The rate of development of the two species is the same.

Egg Size.—The average diameters in millimeters of the three groups of French embryos were 1.90 ± 0.01, 1.81 ± 0.02 and 1.78 ± 0.01 and for the two groups of Belgium embryos 1.67 ± 0.01 and 1.72 ± 0.02. The measurements were made before first cleavage. It would appear that egg diameter in the French *temporaria* is slightly greater than in the Belgium *temporaria* but more data are necessary to establish the point. The average of the five means is 1.77 mm. The average diameter is close to that previously reported[5] for *Rana sylvatica* as 1.9 mm. It would be misleading,

however, to regard this similarity as indicative of close relationship since *Rana pipiens* and *Rana palustris* also have eggs of similar size.

Cross Fertilization Experiments.—Some of the *temporaria* eggs were used in hybridization experiments with *sylvatica*. Mixed sperm of two *sylvatica* males from North Carolina were used in the experiment with the three French *temporaria* females. Approximately 500 eggs from each female were used. In no case did cleavage take place. Results were the same with the two Belgium *temporaria* females and North Carolina *sylvatica* males. In this case approximately 250 eggs from each female were used.

The cross *sylvatica* ♀ × *temporaria* ♂ gave different results. A single female, from Dartmouth, New Hampshire,[7] was used. Four sperm suspensions were used. Two were French *temporaria*, one was a Belgium *temporaria* and the last was a New Hampshire *sylvatica*. The control *sylvatica* ♀ × *sylvatica* ♂ showed normal development. The hybrids were normal in early stages but ceased development in the late blastula stage. No trace of a dorsal lip appeared. The hybrids began to cytolize when the controls were in late stage 14 and cytolysis in the hybrids was complete when the controls were in stage 17. Similar results were obtained when eggs of *Rana pipiens* from Vermont were fertilized with sperm of Belgium *temporaria*. Development was normal during cleavage and blastula stages. No dorsal lip was formed and the embryos remained as arrested late blastulae for 4 days (at 20°C.) and then cytolized. These hybrids resembled the *pipiens* ♀ × *sylvatica* ♂ hybrids previously described[8] in two interesting respects, namely, a deep pitting of the animal hemisphere and marked swelling of the embryos. I have not observed abnormalities of this type in cases where *pipiens* eggs have been fertilized with sperm of other species. Apart from these rather minor points the hybridization experiments do not suggest a close relationship between *temporaria* from western Europe and *sylvatica* from eastern North America.

There is only one species with which European *temporaria* can be crossed and the hybrids develop to adults, namely *Rana arvalis*.[9] In all other reported crosses involving European *temporaria* and other species of the genus *Rana* either there is no development (as in the cross *temporaria* ♀ × *sylvatica* ♂) or development stops in the late blastula (as in the crosses *sylvatica* ♀ × *temporaria* ♂ and *pipiens* ♀ × *temporaria* ♂) or early gastrula stage.

The following interpretation can be given these data. On the basis of structural similarity, identical rates of embryonic development and similar ranges of temperature tolerance it would appear that *Rana sylvatica* is fairly closely related to *Rana temporaria*. (An alternative hypothesis of convergence cannot be ruled out, especially in the case of the embryonic adaptations. Since the species occupy equivalent temperature environments selection could have resulted in two similar physiological systems.

The fact that the rate of development is identical leads me to favor the hypothesis of close relationship.) If a common origin is granted, one would conclude further from the data of geographic distribution that *sylvatica* of eastern America and *temporaria* of western Europe represent the end members of a chain of related species and subspecies extending from western Europe, across northern Asia, through Alaska and Canada, down to the east coast of North America. The terminal members of this chain have reached the species level since divergence has proceeded to the point where gene interchange is no longer possible. Perhaps further experimentation will reveal that hybridization is possible in cases where the populations are not so remote in the geographical sense. If this is the case, the situation would parallel that of *Rana pipiens* where it has been shown that ability to form normal hybrids is correlated with latitudinal distances between the parent populations.[5] A recent report[10] indicates there might be some basis for this suggestion as applied to the *temporaria* group. Kawamura has found that hybrids resulting from the cross *Rana japonica* ♀ × *Rana temporaria ornativentris* ♂ develop to adults. Both of these species are from Japan. Perhaps their ability to hybridize indicates that *japonica* has split off from the main *temporaria* group more recently than has *sylvatica*.

[1] I am indebted to Prof. and Mrs. F. J. Ryan for the French material and to Mr. Philip Grant for that from Belgium.

[2] Moore, J. A., *Genetics*, **31**, 304–326 (1946) and Moore, J. A., *Evolution*, **3**, 1–24 (1949).

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